EFSA Panel on Plant Protection Products and their Residues (PPR); EFSA Scientific Opinion on the science behind the revision of the guidance document on dermal absorption

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SCIENTIFIC OPINION

Scientific Opinion on the Science behind the Revision of the Guidance Document on Dermal Absorption[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2, 3]

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ABSTRACT

This opinion provides the relevant data, evaluations and references that support the criteria proposed in the new draft guidance needed to facilitate assessment of dermal absorption of plant protection products (PPPs) under Directive 91/414/EEC[4] and Regulation (EC) No 1107/2009[5]. The new guidance will be finalised and published only after adoption and publication of this opinion. This opinion has been developed after a public consultation of EFSA on the current guidance document and an outsourced project carried out by the UK Chemicals Regulation Directorate (CRD, 2010). It is not intended to be an exhaustive review but builds on existing documents to which the reader is referred to, and addresses in more detail elements that are specifically relevant for the assessment of dermal absorption of plant protection products. It contains an overview on skin and substance properties and other determinants that have an impact on absorption, a description of important elements in the design of experimental studies and an analysis of available data on dermal absorption of PPPs. The PPR Panel concludes that assessment of dermal absorption in the absence of specific studies can be performed based on default values that the Panel derived from the analysis of the available databases. Elements for a tiered approach are described and commented on. The PPR Panel also concludes that *in vitro* human studies can be used as a stand alone test for assessment of dermal absorption. The Panel indicates some additional requirements to adapt existing OECD test guidelines to the special requirements of PPPs and their formulations. A harmonised reporting of the studies is also considered important. Some areas where further research could usefully be conducted have been identified.

KEY WORDS

Dermal Absorption, Pesticides, Guidance

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4 EC (1991). Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230, 1-290. 19 August 1991.

5 EU, 2009. Regulation (EC) No 1107/2009 of the European Parlament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EC and 91/414/EEC. Official Journal L 309, 1-50. 24 November 2009.

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SUMMARY

Following a request from EFSA, the Panel on Plant Protection Products and their Residues (PPR) was asked to deliver a scientific opinion on the science behind the revision of the guidance document on dermal absorption.

Experience has shown that interpretation of dermal absorption data lacks consistency even when the existing guidance document is used, leading to recurring issues that have provoked extensive debates during the evaluation of plant protection products (PPPs) under Directive 91/414/EEC. In addition to that there are also developments in scientific understanding of dermal absorption processes and new evaluations done in the field by national and supranational bodies (Holmgaard and Nielsen, 2009; WHO, 2006; OECD 2004a, b, c).

The starting point for the present opinion was a public consultation of EFSA on the current guidance document that was launched with the aim of involving stakeholders and to gain information about further issues to be addressed in the opinion. A report on the outcome of the consultation is available on the EFSA website (EFSA, 2009a).

The second preparatory step was an outsourced project carried out by the UK Chemicals Regulation Directorate (CRD) who was asked to provide a proposal for a revision of the guidance document based on literature review and analyses of data taking into account the outcome of the stakeholder consultation. The report from the outsourced project (CRD, 2010) is available on the EFSA website.

The present opinion provides the relevant data, evaluations and references that support the criteria proposed in the revised draft guidance document needed to facilitate evaluations of chemical PPPs under Directive 91/414/EEC and Regulation (EC) No 1107/2009.

It contains (i) an overview on skin and substance properties and other determinants that have an impact on absorption, (ii) a description of important elements in the design of experimental studies and (iii) an analysis of available data on dermal absorption of PPPs that together with (iv) an extensive literature review, constitute the basis for the different default assumptions and the decision criteria given in the draft guidance document.

The responses to the comments obtained in the public consultations are included as an appendix to this opinion. The Guidance constitutes a separate output of the PPR Panel.

The PPR Panel concludes that dermal absorption in the absence of specific studies that are not mandatory under the present regulation can be performed based on default values that the Panel derived from the analysis of the available databases. Depending on availability of studies, elements for a tiered approach have been described and are presented in the proposed guidance document. However, the PPR panel concludes that an *in vitro* human study can be used as a stand alone test to derive dermal absorption values for risk assessment. The Panel underlines the need for an adaptation of existing OECD test guidelines to the specificity of PPPs and their formulations. To this end some additional requirements have been indicated that would help in using the studies for this specific risk assessment.

Blood flow/vasodilatation has been considered to have a minor impact on dermal absorption since they only act after the compound has passed through the epidermis to the vascular dermis. Sweating/skin hydration have been reported to increase dermal absorption as have ambient temperature and high ethanol consumption, but the overall magnitude is reported to be only up to 2- fold. On this basis, the PPR Panel concludes that the influence of these parameters should not impact significantly on dermal absorption of pesticide formulations in normal use.

Mechanical lesions by either tape stripping or abrasion (e.g. by a rotating brush) cause up to >100 fold increase of absorption for compounds that have very low absorption, but this occurs only when the

lesions reach the dermis. In individuals suffering from atopic dermatitis, skin areas not obviously affected by the disease showed about a 2-fold increase in permeability to sodium lauryl sulphate and polyethylene glycols (molecular weight (MW) 150-590). The PPR Panel considers it unlikely that individuals with extensive skin damage would leave the skin unprotected; in case of less extensive skin damage, it would in any case represent a minor fraction of the total exposed area. Based on these data, the PPR Panel concludes that the possible presence of skin damage to a limited skin area, or skin diseases such as atopic dermatitis, will not significantly impact on dermal absorption of pesticide formulations in normal use and that they are covered by the safety factors applied when deriving the acceptable operator exposure level (AOEL). Also minor differences in skin absorption due to age do not necessitate the establishment of different absorption values for children and adults.

Treatment of skin, *in vivo* and *in vitro,* with irritants such as sodium lauryl sulphate (SLS) has been shown to enhance dermal absorption/penetration, the magnitude of the enhancement varying with the compound, the experimental procedure and severity of irritation; in comparison to irritation, simple delipidisation (e.g. by acetone) may increase absorption by 2-fold. Other factors that determine dermal absorption of chemicals, include octanol/water partition coefficient, molecular weight/size, use of detergents/solvents and concentration of the substance. The PPR Panel also notes that there are no good predictors of absorption from pesticide formulations and therefore the PPR Panel is of the opinion that dermal absorption data on plant protection products should be generated on the formulated product, or a closely related product, and on concentrations representative of the spray dilutions as applied to the crop. It is proposed in line with the current regulation on classification ((EC) No 1272/2008) that formulations are considered similar when the content of each safener/synergist and other formulants is within 25% of the actual concentration of the tested formulation, although the Panel is not aware of any scientific evidence for this value. Available data were considered inadequate for any conclusion to be drawn regarding the potential to extrapolate between formulation types; in this case use of default values is suggested.

From the methodological point of view the PPR Panel considers that:

I. The use of data generated following exposures of human torso/breast and upper leg skin *in vitro* will provide realistic dermal absorption values for use in exposure modelling.

II. The use of dermatomed (split-thickness) skin is recommended because both flux and % absorption can be calculated and used for risk assessment, whereas with full-thickness skin only % absorbed can be used.

III. Solid material should be moistened with a minimal volume of vehicle (e.g. water or physiological saline, no organic solvents) to make a paste.

IV. In *in vitro* studies the receptor fluid should not be a rate-limiting element in absorption because of an insufficient solubility of the material under test. In current practice, the expected concentration of the active substance in the receptor fluid should not be higher than 10% of the maximal solubility of the product in the given receptor fluid. Moreover, the receptor fluid should not affect the integrity of the barrier function of the skin.

V. The total amount of a chemical penetrating during a certain time period is not very informative on its own unless most of the compound has been already absorbed. This is defined as 75% absorption taking place in 50% or less of the sampling time.

VI. In order to mimic operator/worker in use conditions, exposure time should be 6 - 10 hours, after which the compound should be swabbed.

VII. Sampling time should last 24 hours and over 75% of the absorption should occur within half of the duration of the total sampling period: in such a case indication of the basis for

calculation of absorption, including considerations of the amount of substance present in the *stratum corneum*, are provided.

VIII. The PPR Panel is of the opinion that both % absorbed and maximum flux are parameters that can be used to compare dermal absorption for instance between species of skin areas, the flux being independent from the recovery of the substance. The maximum flux can be calculated during the first hours, i.e. the first 2 hours (or less if absorption is very rapid) of the linear part of the curve correlating amount absorbed with time.

IX. Tape strips should be analysed separately. The PPR Panel follows the general approach used within EFSA Pesticide Risk Assessment and Peer Review (PRAPeR) whereby the first 2 tape strips are assumed to represent material that will not become bioavailable. The Panel notes that it is very difficult to standardise the tape stripping technique and is not aware of any scientific evidence for the notion that specifically the outer two tape strips can be regarded as non-bioavaliable. Accordingly, this number of two is applied in the guidance only on the basis that it is established practice in current risk assessment of PPP's within in the EU. Only if absorption is essentially complete at the end of the study (75% occurring within half of the study duration) all tape stripped material can be excluded.

X. The substance remaining in the skin at the end of exposure should be considered as absorbed/absorbable after removal by washing that should be representative of normal hygiene practices e.g. an aqueous soap solution.

The PPR Panel considers that skin metabolism will not alter the calculated absorption significantly as the metabolically active cells are below the *stratum corneum* and therefore the main barrier to absorption has to be passed before any metabolism can occur. In addition, the determination of skin absorption for risk assessment usually considers the total percentage penetration of a compound into and across the skin.

Since according to EU Regulation (EC) No 1107/2009 data from human volunteer studies cannot be used to perform risk assessment, only issues related to studies in non-human primates are discussed although most of the considerations will also apply to studies in humans. There are difficulties in such studies relating to the determination of the overall mass balance (e.g. recoveries and carcass and application site residues). It is important to underline the need to cover possible metabolites or marker compound (or compounds) if non-radiolabelled compounds are used that allow back extrapolation to absorbed material based on the amount in urine, faeces and exhaled air.

The PPR Panel is of the opinion that in any case, experiments with non-human primates are difficult to carry out and to be interpreted. Therefore, their use is discouraged, and the recommendations provided in the guidance solely refer to already existing studies.

Techniques used to determine the relationship between excreted and absorbed material generally involve comparison of excreted material following oral, intravenous or dermal dosing. The dermal study should include a sampling duration long enough to confirm that excretion is essentially complete. Indications for calculations are provided indicating the issues related to first-pass effect, incomplete absorption or extensive biliary excretion when the oral route is used for comparison. The alternative approach of determining the ratio of the area under the curve (AUC) from intravenous or oral dosing with that from an equivalent dermal dose is also presented. In any case, it is the responsibility of the notifier to present a justification for the analytical method used and how the recovered material relates to the amount actually absorbed.

The Panel also identified the need for a harmonised reporting of the studies that will help in taking decisions that are consistent across time and MS.

In summary, the approaches recommended in this opinion are based on a combination of methods and default values. Some of these are based on evaluation of data but others are based on recommendations in other regulations/guidance for which the Panel could not find clear scientific support. Overall, taking account of the uncertainties involved, it is the Panels opinion that the dermal absorption estimate will be sufficiently protective.

The PPR Panel recommends that a new guidance document on dermal absorption should be adopted along the lines of that set out in a separate document.

The new guidance will be finalised and published only after adoption and publication of this opinion.

The guidance should be reviewed periodically and if appropriate revised as well as when relevant new data becomes available.

Further research could usefully be conducted on: (i) default values, (ii) (Q)SAR and read-across, (iii) skin lesions and diseases (relevance and occurrence), (iv) evaluation of the proper experimental conditions to mimic use of PPE, (v) extrapolation between formulation types, (vi) identification of the most appropriate approach to define dermal absorption values for re-entry workers (dilution versus concentrate) and (vii) studies on dermal absorption of nanoformulations.

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BACKGROUND AS PROVIDED BY EFSA

The compilation and revision of Guidance Documents to assist the implementation of Council Directive 91/414/EEC was originally the responsibility of the European Commission. This remit has now been transferred to EFSA.

In 2006, EFSA has consulted Member States on their priorities for development and revision of such Guidance Documents. In response some Member States expressed a wish for an update or a revision of the Guidance Document on Dermal Absorption. The PRAPeR Unit acts as an interface between the needs of peer review and the development of guidance by the PPR Panel

Information on dermal absorption is a data requirement for plant protection products laid down in Annex III of Directive 91/414/EEC.

Currently, a tiered approach as described in the Guidance Document on Dermal Absorption (Sanco/222/2000 rev. 7, adopted in 2004) is followed for the assessment of dermal absorption of plant protection products. In the practical use of this guidance document during the peer review of Draft Assessment Reports of plant protection products, some issues not sufficiently covered in the current guidance document have been raised as points of discussion (e.g. tape stripping).

With implementation of the revision of Directive 91/414/EEC, modifications are foreseen also to the data requirements relating to dermal absorption, which are not reflected in the current guidance.

In addition, recent re-evaluations, either still on-going (OECD, Draft Guidance Notes for the Estimation of Dermal Absorption Values, Draft 26 May 2008) or already published (Environmental Health Criteria 235, Dermal Absorption, 2006), have been carried out in which refined or alternative approaches have been introduced that should be evaluated by the PPR Panel with regard to the needs of pesticide risk assessment.

Furthermore, an investigation of available data/information from practical cases (peer review of PPPs) could be a basis for revision of the guidance document (a procurement might be needed for that task).

TERMS OF REFERENCE AS PROVIDED BY EFSA

Should the current Guidance Document on Dermal Absorption (Sanco/222/2000 rev. 7) be revised in view of the draft data requirements in the revision of Annexes II and III of Dir. 91/414/EEC, relevant recently updated documents from other scientific bodies (e.g. OECD and WHO) and relevant opinions of the PPR Panel.

The following issues have been identified in particular. However, the scope of the opinion should not necessarily be limited to these:

a. Should the tiered approach for the assessment of dermal absorption as proposed in the current guidance document be revised or amended?

b. Should the minimal data requirements in the current guidance document for the individual stages of the tiered approach be modified?

c. The PPR panel is asked to give advice regarding the interpretation of in vitro and in vivo test results obtained using tape stripping techniques.

d. Should the approach to the use of flux versus mass balance in the current guidance document be reconsidered, taking into account the recommendations given in the Draft Guidance Notes for the Estimation of Dermal Absorption Values of the OECD?

ASSESSMENT

1. Introduction

Dermal absorption is a critical element in the risk assessment of plant protection products for operators, workers, bystanders and residents. Authorisation of active substances according to Directive 91/414/EEC and the new Regulation (EC) 1107/2009 is possible only if the products containing them can be used with acceptable risk to humans.

Until to date dermal absorption of PPPs is either estimated on the basis of physico-chemical properties of the active substance or on results from experimental studies. Internationally agreed test guidelines exist for the performance of dermal absorption studies both *in vivo* and *in vitro* (OECD 2004a, b, c). These guidelines are designed to cover a range of chemicals and are not focussed on the assessment of PPPs. They also give only limited guidance on the interpretation of the results obtained.

In order to give further support for the assessment of dermal absorption the *Guidance Document on Dermal Absorption* (Sanco/222/2000 rev. 7, 19 March 2004) is currently in use and was conceived by Commission services in co-operation with EU Member States.

Experience has shown that interpretation of dermal absorption data lacks consistency even when the guidance document is used leading to recurring issues that are provoking extensive debates during the evaluation of PPPs under Directive 91/414/EEC. In addition to that there are also developments in scientific understanding of dermal absorption processes and new evaluations done in the field by national and supranational bodies (Holmgaard and Nielsen, 2009; WHO; 2006).

Since it is essential that guidance documents are both contemporary and useful, EFSA's Executive Director requested the PPR Panel to carry out a scientific opinion on the science behind revision of the current guidance document and a revised guidance document.

The starting point for the present opinion was a public consultation of EFSA on the current guidance document that was launched with the aim of involving stakeholders and to gain information about further issues to be addressed in the opinion. A report on the outcome of the consultation is available on the EFSA website (EFSA, 2009a).

The second preparatory step was an outsourced project carried out by the UK Chemicals Regulation Directorate (CRD) who was asked to provide a proposal for a revision of the guidance document based on literature review and analyses of data by duly taking into account the outcome of the stakeholder consultation. The report from the outsourced project is available on the EFSA website (CRD, 2010).

The draft opinion and the draft guidance were then posted for another public consultation before adoption by the PPR Panel.

The present opinion provides the relevant data, evaluations and relative references that support the criteria proposed in the revised draft guidance document needed to facilitate evaluations of PPPs under Directive 91/414/EEC and Regulation (EC) No 1107/2009. The opinion and the guidance document are intended for evaluation of dermal absorption of chemical PPPs only. The opinion contains an overview on skin and substance properties and other determinants that have an impact on absorption, a description of important elements in the design of experimental studies and an analysis of available data on dermal absorption of PPPs that together with an extensive literature review constitute the basis for the different default assumptions and the decision criteria given in the draft guidance document.

The responses to the comments obtained in the public consultations are included as an appendix to this opinion. The guidance constitutes a separate output of the PPR Panel.

2. The skin and properties affecting dermal absorption

Detailed descriptions of the skin and the process of dermal absorption are covered extensively in reference texts (e.g. WHO, 2006; Marzulli & Maibach, 1996; Zhai et al., 2008). The skin is a protective organ made up of several distinct layers of cells and associated features such as hair follicles, sweat glands and sebaceous glands. The skin can be considered to have an outer region (epidermis) and an inner region (the vascularised dermis). The epidermis consists of 4 layers. The main barrier to absorption of chemicals is the outermost layer of the epidermis, the *stratum corneum*, which is typically made of 15 – 20 layers of non-viable cells. The *stratum corneum* varies in thickness with anatomical site and species (10 – 600 μm) which, with other factors such as hair follicle density, can influence dermal absorption.

2.1. Human versus laboratory animal skin

In general human skin is less permeable than that of laboratory animals with pig or non-human primate skin being considered the best predictive model for human percutaneous absorption (Monteiro-Riviere, 2008; WHO, 2006; Holmgaard and Nielsen, 2009). However, the rat is the preferred species for *in vivo* studies because of consistency and the fact that more extensive and complete data can be collected in this species *in vivo* (e.g. excreta and carcass, see also Section 3.8.). The PPR Panel concluded that human *in vitro* data can be used as stand alone data to predict dermal absorption (see also section 4.1.3).

2.2. Anatomical sites influence absorption

Different anatomical sites in humans display a hierarchy of absorption: scrotum > forehead > torso & arms > palms and soles of feet (e.g. Weltfriend et al, 1996; Maibach et al., 1971).

Table 1: Comparison of dermal absorption at different anatomical sites (based on Maibach et al., 1971) in human volunteers. Normalised to abdomen = 1.0.

Site	Malathion (4ug/ml in acetone)	Parathion (4ug/ml in acetone)
Abdomen	1.0	1.0
Forearm	0.7	0.5
Palm	0.6	0.6
Ball of foot	0.7	-
Back of hand	1.3	1.1
Inside of elbow	-	1.5
Scalp	-	1.7
Jaw angle	-	1.8
Forehead	2.5	2.0
Arm pit	3.1	3.5
Scrotum	-	5.5

Dermal absorption studies in humans normally use skin from the back (*in vivo*) or breast/abdomen and upper leg (*in vitro*). Operator exposure models are underpinned by data from whole body exposures, taking account of the relative areas of the high dermal absorption sites it is considered that the use of data generated following exposures of torso/breast skin will provide realistic dermal absorption values for use in exposure modelling.

2.3. Physiological parameters

Physiological parameters such as blood flow/vasodilatation and sweating/skin hydration have been reported to influence dermal absorption. However, data on the impact of blood flow/vasodilatation are

inconsistent. They only act after the compound has passed through the epidermis to the vascular dermis, hence they appear to vary with the physical and chemical parameters of the molecule (WHO, 2006; Gordon and Leon, 2005; Tanner and Marks, 2008; Monteiro-Riviere, 2008). Sweating/skin hydration have been reported to increase dermal absorption (Gordon and Leon, 2005; Williams et al., 2004; WHO 2006) as has ambient temperature but the overall magnitude is reported to be < 2 fold (Gordon and Leon, 2005) except when occlusion was applied causing up to 9-fold increases (reviewed by Kezic and Nielsen, 2009). It can be argued that contamination by pesticide formulations/dilutions under the gloves may mimic occlusive conditions. However, good hygienic practices include washing of hands and change of gloves soon after occurrence of such contamination.

It has also been shown in rats that ethanol consumption increases dermal absorption, however, in order to obtain a 2-fold increase a daily intake of 6 g ethanol/kg bw was needed (Brand et al., 2006).

Based on these data, it is concluded that the influence of these parameters should not impact significantly on dermal absorption of pesticide formulations in normal use.

2.4. Skin irritation, damaged skin and skin diseases

Since the principal barrier of the skin is provided by the *stratum corneum*, superficial lesions of the skin may have a significant effect on dermal absorption.

Treatment of skin, *in vivo* and *in vitro* with irritants such as sodium lauryl sulphate (SLS) has been shown to enhance dermal absorption/penetration, the magnitude of the enhancement varying with compound, the experimental procedure and severity of irritation. This varied from 2-4 fold in the case of mild irritation (e.g. 5% SLS for 4 h) to about 46-fold in the case of more severe irritative dermatitis (e.g. SLS 1% for 24 h) for compounds that have a very low penetration through normal skin such as salicylic acid (Benfeldt et al., 1999; Benfeldt and Serup, 1999; Jakasa et al., 2006; Nielsen, 2005; Kezic and Nielsen, 2009; Nielsen and Holmgaard, 2009). The PPR Panel notes, that a sensitisation reaction may also enhance dermal absorption.

In comparison to irritation, simple delipidisation (e.g. by acetone) may increase absorption by 2-fold if compounds have a low penetration rate (Benfeld et al., 1999). More marked effects can be observed under more stressing conditions (e.g. occlusion and high concentration of solvent): in general, the effect is less evident with moderately lipophilic compounds (see Kezic and Nielsen, 2009 for review).

Mechanical lesions by either tape stripping or abrasion (e.g. by a rotating brush) cause up to >100 fold increase of absorption, but this occurs only when the lesions reach the dermis (Akomeah et al., 2008; Benfeldt and Serup, 1999; Benfeldt et al., 1999; Morgan et al., 2003; Kezic and Nielsen, 2009; Holmgaard and Nielsen, 2009).

The PPR Panel noted that commercial PPPs do/may contain solvents and surfactants. For these reasons, formulations need to be tested in order to include the effects of solvents and surfactants. Given the fact that such effects also depend on their concentration guidance is given on how to consider different formulations as similar in this respect (see Section 2.6.).

In addition, it is considered unlikely that individuals with extensive skin damage would leave the skin unprotected; in case of less extensive skin damage, it would in any case represent a minor fraction of the total exposed area that is used in models of operator exposure. It should also be underlined that the damage needs to be as deep as the dermis to significantly modify penetration. In addition, operators and workers who are predicted to have a higher exposure than bystanders and residents are expected to wear gloves if their skin is damaged.

Studies have also been performed on subjects with skin disease. In particular in individuals suffering from atopic dermatitis, skin areas not obviously affected by the disease showed about a 2-fold increase

in permeability to sodium lauryl sulphate and polyethylene glycols (MW 150-590, reviewed by Kezic and Nielsen, 2009).

Based on these data, it is concluded that the possible presence of minor or even severe skin damage to a limited skin area will not significantly impact on dermal absorption of pesticide formulations in normal use and that they are covered by the safety factors applied when deriving the AOEL. The same considerations apply to skin diseases such as atopic dermatitis.

2.5. Age related differences in dermal absorption

There are no systematic data on age-related differences in dermal absorption. However, scattered data show that the number of layers of the *stratum corneum* increases with age in certain areas (e.g. facial skin and skin on the back) but not in other areas (Ya-Xian et al., 1999; Kashibuchi et al., 2002; Tagami, 2008). These changes are associated with changes in transepidermal water loss (TEWL) which show on average a decrease in elderly people of 2-3 fold when compared with children (Ya-Xian et al., 1999; Tagami, 2008). TEWL is considered to be an index of skin permeability (Holmgaard and Nielsen, 2009). It should also be noted that the same authors report a high inter individual variability such that values in old adults overlap with those of young children.

In conclusion, possible minor differences in skin absorption due to age appear to be limited to certain skin areas only and do not call for any correction factor or any specific default figures to be applied.

2.6. Determinants of dermal absorption

Dermal absorption of chemicals can occur by several mechanisms (WHO, 2006; Wilkinson et al., 2006) and is related to several (combinations of) factors, including:

- Octanol/water partition coefficient of the substance (log Pow, Nielsen et al., 2004). However this does not appear to apply to absorption from pesticide formulations (see Figs. 1 and 2 of the CRD Report). It is also of note that log Pow can change with pH (Nielsen et al., 2009), and that pesticide formulations cover a range from pH<3 to pH>10 (see section 4 for a more detailed discussion).

- Molecular size of the substance (Nielsen et al., 2009; WHO, 2006). In a number of analyses and models, molecular weight has been substituted for molecular size. This relationship between molecular weight and size is reasonable within a homologous series but does not necessarily apply to a heterogeneous group of chemicals such as pesticide active substances (Nielsen et al., 2009). A link between molecular weight and dermal absorption does not appear to apply to absorption from pesticide formulations (see Fig. 4 and 5 of the CRD report, and section 4 for a more detailed discussion).

- The ionisation state of a molecule. Ionised molecules have lower absorption than the corresponding non-ionised forms, where the permeability coefficient is lower by 1-2 orders of magnitude (WHO, 2006).

- An absorbable (organic) solvent drags a dissolved lipophilic substance with it (WHO, 2006).

- If a lipophilic substance partitions into a non-absorbed solvent rather than into the *stratum corneum*, absorption will be reduced (WHO, 2006).

- Absorption of particles of a lipophilic substance from a suspension in an aqueous vehicle is reported to be lower than from a solution (van de Merwe and Riviere, 2005; Nielsen et al., 2009).

- Changing the solvent can alter the partitioning of the active substance between the *stratum corneum* and vehicle, which could increase or decrease dermal absorption. If the solvent is absorbed the active substance might be drawn through with it. Surfactants could alter the barrier properties of the *stratum corneum* and/or produce an irritant reaction that could increase dermal absorption. (Nielsen, 2005; van de Merwe and Riviere, 2005)

- In general, dermal absorption of a chemical is higher (in terms of the percentage of the applied material) following the application of a low concentration as compared to a higher concentration (Liu and Kim, 2003; Buist et al., 2009; see Annex 5 and Figs. 1 and 2 of the CRD Report). Dermal absorption is generally a simple diffusion process that depends on concentration gradients but can also be limited by the speed at which individual molecules can diffuse (Liu and Kim, 2003; Holmgaard and Nielsen, 2009). However, in case of known irritants and volatiles, this relationship may be masked by local toxicodynamic effects. Irritation may decrease functioning of the skin barrier and evaporation may prevent saturation of the absorption process (Buist et al., 2009). Thus, general rules are difficult to define since the effect of dilution will also depend on other parameters such as characteristics of the molecule, solvents and skin conditions.

- Detergents can act by enhancing the maximal flux with no effect on lag-time, or by decreasing lag-time with no effect on flux (Holmgaard and Nielsen, 2009).

For these reasons, ideally, dermal absorption data on plant protection products should be generated on the formulated products or closely related products and on concentrations representative of the spray dilutions as applied to the crop including the greatest spray dilution (lowest concentration, see also Section 4). In current practice, formulations are considered similar when the content of each solvent/surfactant/detergent/emulsifier is within 25% of the actual concentration of the tested formulation. This is a proposal that is based on the bridging principle for changes in the composition of a mixture that requires reconsideration of classification (see Table 1.2 of Annex I to Regulation (EC) No 1272/2008) and on the observation that major modifications are required to obtain significant changes in absorption. The Panel is not aware of any scientific evidence for the value of 25%, so this value is followed only on the basis that it is established practice under Regulation (EC) No 1272/2008.

The potential to extrapolate between formulation types was discussed, but the data that were available were considered inadequate for any conclusion to be drawn. Therefore, no guidance could be given on how to determine dermal absorption for different formulation types other than using default values.

3. Elements of study design

3.1. Preparation of skin samples

Different methods can be used to prepare skin samples (WHO, 2006). These include:

- Full-thickness skin, incorporating the *stratum corneum*, viable epidermis, and dermis: This is normally used for mechanistic studies and should be used in other studies only when justified (OECD, 2004c; WHO, 2006).

- Dermatomed (split-thickness) skin, in which the lower dermis has been removed: A dermatome (a surgical instrument for cutting skin grafts) is used to obtain skin samples of uniform shape and thickness (Steiling et al., 2001).

- Epidermal membranes, comprising the viable epidermis and the *stratum corneum*: Preparation of an epidermal layer by separation of the epidermis from the dermis using heat is effective for non-hairy skin. The most frequently used method is by submersion of full-thickness skin in water heated to 60 °C for approximately 45 seconds. The epidermal and dermal layers can be

pulled apart with forceps, but the metabolic viability of the skin is destroyed (Bronaugh, 2004; US EPA, 2004). Chemical or enzyme treatments can also be used to separate the dermis from the epidermis. The use of epidermal membranes may in some cases overestimate human *in vivo* skin absorption because of insufficient barrier function (van de Sandt et al., 2000; SCCNFP; 2003; SCCS, 2010).

- *Stratum corneum* alone: This tissue is prepared from epidermal membranes by enzyme treatment with trypsin. *Stratum corneum* membranes are primarily used for mechanistic studies and partition coefficient determination.

Various guidelines and documents indicate that the skin samples that may be used for *in vitro* studies are split-thickness (200–400/500 µm, OECD, 2004c; US EPA, 2004; WHO, 2006; Wilkinson et al., 2006) or, when justified, full-thickness (500–1000 µm) skin preparations (OECD, 2004c). The main difference of full-thickness skin from difference split-thickness skin is the amount in the receptor fluid, as split-thickness membranes tend to have significantly lower levels of residual material (Wilkinson et al., 2006; WHO, 2006; OECD, 2004c) and flux, with the sum of receptor fluid plus skin sample being similar for both preparations (Wilkinson et al., 2006; Vallet et al., 2007). Therefore by including all material remaining in the skin sample, a dermal absorption value can be obtained in both cases. However, any calculated fluxes with full-thickness skin should not be used. Animal and human skin can be stored for up to 1 year at 20 °C (Bronaugh et al., 1986; Steiling et al., 2001). Frozen stored skin may not be suitable for some metabolism studies. Barrier integrity should be evaluated after storage.

3.2. Moistening

Solid material should be moistened with a minimal volume of vehicle (e.g. water or physiological saline) to make a paste. This is to mimic sweat on the skin or (semi) occlusive conditions under clothing. Organic solvents should normally not be used. Under experimental occlusive conditions it can be assumed that a reasonable match to actual exposures can occur even without previous moistening of the material, except for granules.

3.3. Receptor fluid

In *in vitro* studies the receptor fluid should not be a rate-limiting element in absorption because of an insufficient solubility of the material under test. Moreover, the receptor fluid should not affect the integrity of the barrier function of the skin. Literature does not provide adequate quantitative data that allow the definition of precise criteria in the choice of the proper receptor fluid. However, while normal saline is sufficient for water soluble compounds, for lipophilic substances, depending on the expected concentration, mixtures containing solvents (e.g. ethanol/water) might be required. It should also be born in mind that such mixtures might damage the skin barrier leading to an overestimation of the absorption. In current practice, the expected concentration of the active substance in the receptor fluid should not be higher than 10% of the maximal solubility of the product in the given receptor fluid (OECD Guidance Document 28).

3.4. Kinetic parameters

Percutaneous absorption includes permeation through the epidermis and uptake by the capillary network at the dermal-epidermal junction. Permeation through the *stratum corneum* is basically a diffusion process and for many compounds it is the rate-limiting barrier. However, diffusion through the hydrophilic epidermis and dermis might be the rate limiting factor for very lipophilic compounds, as is insufficient blood flow. A detailed description of kinetic parameters, their measurement and significance can be found in WHO (2006) and Holmgaard and Nielsen (2009). In this document, only points relevant for risk assessment of pesticides and pesticide formulations are briefly discussed. The kinetic of the penetration of a compound through a membrane, such as the skin, can be described as in Fig. 1.

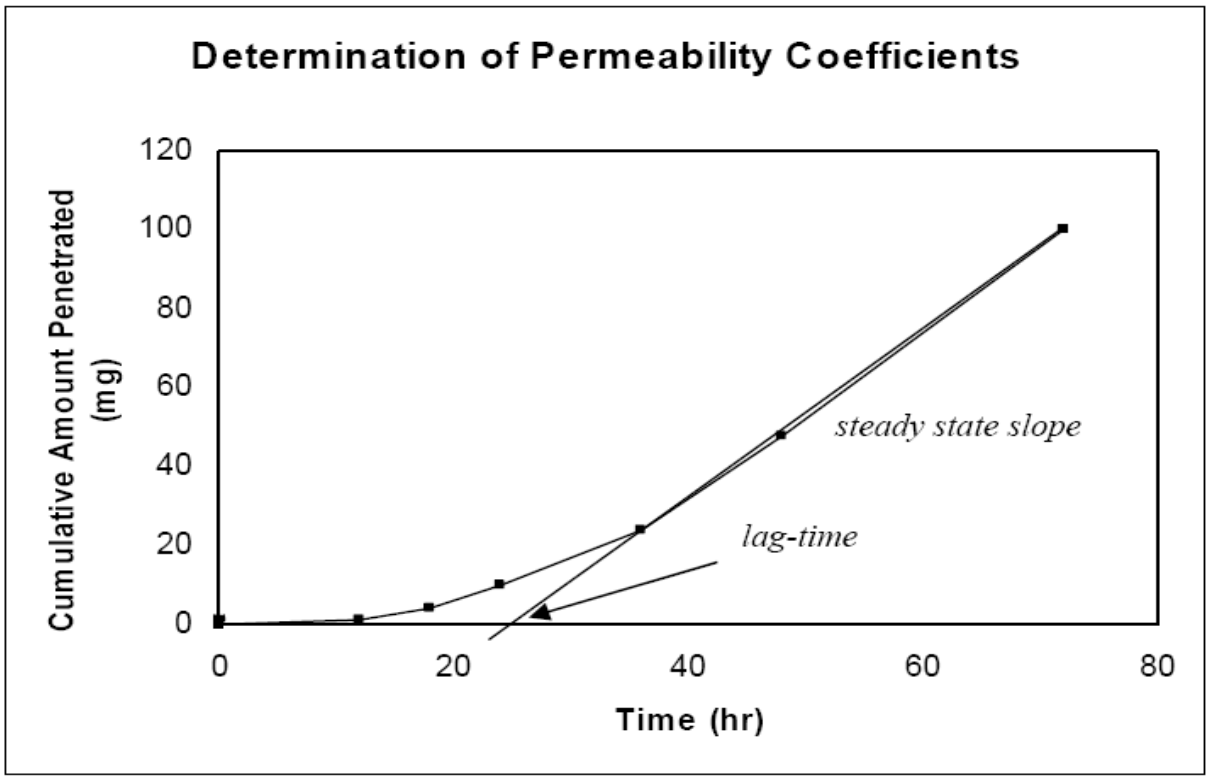

Figure 1: Kinetics of the penetration of a compound through a membrane (from Holmgaard and Nielsen, 2009). The slope of the linear part of the graph of the cumulative amount penetrated as a function of time represents the steady-state flux. The lag-time is the intercept of the linear portion of the graph.

The slope of the linear part of the graph of the cumulative amount penetrated as a function of time represents the steady-state flux. The lag-time is the intercept of the linear portion of the graph. Different combinations of flux and lag-time may cause identical amounts of a chemical to cross the dermal barrier at a certain point in time. Thus, the total amount of a chemical penetrating during a certain time period is not very informative on its own unless most of the compound has already been absorbed. This is defined as 75% absorption taking place in 50% or less of the sampling time (see section 3.5.). This is especially relevant in relation to short term exposures (Holmgaard and Nielsen, 2009).

The maximum flux through the skin defines the highest exposure risk for a chemical when the chemical is present at the solubility limit; dermal absorption would be no higher than the maximum flux, except when the compound can alter or damage the skin or when solutions are supersaturated (WHO, 2006). The flux is dependent on the concentration of the chemical and when experiments occur at finite dose, it decreases with the decrease of the concentration of the chemical on the skin. However, the maximum flux of the linear part of the curve can be calculated during the first hours, normally the first 2 hours, or less if absorption is very quick. The finite (and the intermediate semi-finite) dose experiments also allow the determination of total absorption as % of the applied dose (WHO, 2006). These conditions usually apply to both actual human and experimental exposure to pesticides and pesticide formulations. Therefore, both % absorbed and flux are parameters that can be used to compare dermal absorption e.g. between species of skin areas, the flux being independent from the recovery of the substance.

3.5. Exposure and sampling duration

In order to mimic operator/worker in use conditions, exposure time should be 6-10 hours, after which the compound should be swabbed. Sampling should be done up to 24 hours in *in vitro* tests and for a minimum of 96 hours in *in vivo* tests.

If a dermal absorption study with rat or human skin samples has been well performed (see section 5 and OECD TG 428) the dermal absorption should be calculated on the following basis using mean values:

e.g. when:

- the sampling period is 24 hours or longer

  and

- over 75% of the total absorption (material in the receptor fluid at the end of the study) occurred within half of the duration of the total sampling period,

  then

  absorption = receptor fluid + receptor chamber washes + skin sample (excluding all tape strips)

  The reason for this approach is that 75% represents two half-lives. If 75% of the absorption occurs within half of the duration, the total study duration should cover at least four half-lives (i.e. over 93% of the potential absorption under normal exponential conditions). The residual material in the lower layers of the skin sample is included as literature (WHO, 2006; Holmgaard and Nielsen, 2009; OECD, 2004c) indicates that *in vitro* dermal absorption studies provide a good (and conservative) model for human *in vivo* absorption when the skin residue is included. Under the circumstances described, the biological relevance of the material in the *stratum corneum* is considered to be minimal. The argument is that if most of the absorption has finished within the first half of the study what is left in the *stratum corneum* at the end is unlikely to make a significant contribution.

3.6. Tape stripping

Tape stripping is the application of adhesive tape to the area of skin that was exposed to a chemical at the end of a dermal absorption study. An even (often predetermined) pressure is applied to the tape before it is removed, taking a layer of the *stratum corneum* with it. The tape strip is then analysed to determine the amount of chemical that was present in the removed *stratum corneum*. The process is repeated to remove sequentially lower layers of the *stratum corneum*. It has been proved to be very difficult to standardise the technique in order to have a constant fraction removed because many parameters influence the outcome including for instance pressure applied, type of tape, peeling force for removal, anatomical site, age and vehicle used (WHO, 2006). Several methods for measuring the amount of *stratum corneum* removed after each tape-strip have been suggested (WHO, 2006), but none have been validated or appears to be easily applicable. If the tape strips are analysed separately a profile of the chemical within the *stratum corneum* can be determined. Material in the first tape strips (outer layers of the *stratum corneum*) might not be absorbed but lost via desquamation. Material in the lower layers of the *stratum corneum* might also be lost via desquamation but could also penetrate into the dermis. Determination of the likely fate of material in the *stratum corneum* is influenced by several factors including duration of the study and the absorption versus time profile (these are considered below). In general the first two outer layers are considered equivalent to the *in vivo* layers that would be sloughed off.

It is general practise in EFSA Pesticide Risk Assessment Peer Review (PRAPeR) that the first 2 tape strips will represent material that will not become bioavailable provided the application site is swabbed to remove the test material in time before study termination. This applies to both *in vitro* and *in vivo* studies. This is consistent with the approach described in the Manual of Technical Agreement of the Biocides Technical meeting (EC, 2010). The Panel notes that it is very difficult to standardise the tape stripping technique and is not aware of any scientific evidence for the notion that specifically the outer two tape strips can be regarded as non-bioavaliable. Accordingly, this number of two is applied in the guidance only on the basis that it is established practice in current risk assessment of PPP's within in the EU.

Only if absorption is essentially complete at the end of the study (75% of the absorption occurring within half of the study duration) can all tape stripped material be excluded. This applies to both *in vitro* and *in vivo* studies.

For *in vivo* studies where there is evidence that absorption is nearing completion but has not met the upper 75% criterion, material in tape strips 3 onwards can be excluded from the absorbed material if the evidence indicates it is not bioavailable. This should be assessed on a case-by-case basis.

3.7. Washing after exposure (swabbing)

The substance remaining in the skin at the end of exposure should be considered as absorbed/absorbable. However, parts of it can be removed by washing. The percentage of compound that can be removed by washing depends on its characteristics and on the liquid/solution used for washing. Therefore, based on the assumption that operators and workers will wash after the end of the exposure, it is considered appropriate that after the end of experimental exposure, the skin is washed, and the characteristics of the liquid/solution will be indicated in the report. If washing is not performed, an overestimate of the absorption is likely to occur. The cleansing agent should be representative of normal hygiene practices, for instance an aqueous soap solution.

3.8. Metabolic activity of the skin

Skin metabolism of exogenous chemicals can occur mainly in the epidermis layer of the skin and pilosebaceous glands. Metabolic processes can alter the absorption of a chemical through the skin *in vivo* but much less so *in vitro*. However, it is considered that this will not alter the calculated absorption significantly as the metabolically active cells are below the *stratum corneum* and therefore the main barrier to absorption has to be passed before any metabolism can occur. In addition, the determination of skin absorption for risk assessment usually considers the total percentage penetration of a compound into and across the skin. Therefore, metabolism is not a critical factor in interpreting the data and (the lack of) metabolic activity *in vitro* would not underestimate the total dermal absorption. Literature data (reviewed in WHO, 2006 and Holmgaard and Nielsen, 2009) indicate that *in vitro* dermal penetration through rat and human skin is always higher than *in vivo*.

3.9. Studies on humans and non-human primates

Since, according to EU Regulation (EC) No 1107/2009 data from human volunteer studies cannot be used to perform risk assessment, only issues related to studies in non-human primates are discussed here. However, most of the considerations will also apply to studies in humans.

There are difficulties with performing dermal absorption studies in non-human primates which can impact on the interpretation. If non-human primates were not sacrificed at the end of the study issues relating to recoveries, carcass and application site residues should be considered.

A minimum group size of 4 should be used (this in line with OECD TG 427); if smaller numbers are used then the highest result rather than the mean should be chosen. The application site should be selected so that it gives a realistic value of dermal penetration (e.g. forearm, torso, forehead); applications to the palm of the hand can give an unrepresentatively low value and should not be used without appropriate correction.

3.9.1. Correcting for mass balance

The main difficulty in carrying out dermal absorption studies in non-human primates is to determine the overall mass balance. If non-radiolabelled material is used the analyses need to cover possible metabolites or marker compound(s) have to be used that allow back extrapolation to absorbed material based on the amount in urine, faeces and exhaled air. Also it is usually not possible to determine the residue in the dermis at the application site or distributed within the body (this might be possible with techniques such as positron emission tomography (PET)).

Techniques used to determine the relationship between excreted and absorbed material generally involve comparison of excreted material following oral or intravenous and dermal dosing. The dermal study should include a sampling duration long enough to confirm that excretion is essentially complete.

For example, if 25% of an intravenous dose is detected in excreta using a particular analytical technique and 5% of a dermal dose is detected in excreta then the dermal absorption can be considered to be 20% (5 x 100/25).

Comparison with an oral dose becomes more complicated if there is an extensive first pass metabolism, incomplete absorption or extensive biliary excretion. A first tier approach would be to assume 100% oral absorption and determine the ratio of the amount detected in urine in the dermal study with the amount in urine from the oral study.

Alternative approaches to measuring excreta are to take blood samples and determine the ratio of the area under the curve (AUC) from intravenous or oral dosing with that from an equivalent dermal dose, the default assumption being 100% absorption from the oral route.

e.g. (AUC dermal/AUC oral) x 100% = dermal absorption.

It is the responsibility of the notifier to present a justification for the analytical method used and how the recovered material relates to the amount actually absorbed. Alternatively as a conservative approach it can be assumed that all material not recovered in the skin washes plus first two tape strips (if performed) is absorbed.

The PPR Panel is of the opinion that in any case, experiments with non-human primates are difficult to carry out and to be interpreted. Therefore, their use is discouraged, and the recommendations provided in the guidance solely refer to already existing studies.

4. Analysis of available data on dermal absorption of plant protection products

4.1. Analysis of PRAPeR[6] discussions on dermal absorption

Details of dermal absorption decisions on 104 active substances discussed at EFSA PRAPeR meetings were investigated. Complete results and the basic details of active substance, formulation type, agreed values, proposed values in the Draft Assessment Report (DAR), study types and key aspects of the

[6] Pesticides Risk Assessment Peer Review (carried out under the responsibility of EFSA's Pesticides Unit in the EU)

conclusions can be found in the CRD Report (Section 4). The PPR Panel noted that the evaluated studies have not been performed according to standard protocols and evaluated against standard criteria: therefore, conclusions reached by the analysis are not robust when comparisons across active substances and formulated products are performed. For a number of compounds the basis for the conclusion on dermal absorption was not clear and for others documentation of the study results was limited. However, the data did permit some patterns to be determined as described in Table 2.

Table 2: Summary of decisions taken on dermal absorption during PRAPeR meetings

Number of compounds	Absorption value	Notes
46	Proposed[1] and supported	-----
10	Proposed[1] but not supported	Different evaluation of bioavailability of residue at application site
10	Proposed[1] but not supported	Different interpretation of *in vitro* correction ratio
31	Evaluated according to the "triple pack" approach	-----
13	100% default value applied	No reliable data available
8	10% default value applied	No reliable data available
5	No value set	
7	Use of oral absorption value	Sometimes directly, sometimes supportive

[1]by the Rapporteur Member State

4.1.1. Analysis of dermal absorption values based on study data

Analysis of the agreed values based on data from dermal absorption studies for 63 active substances shows that for concentrates a highest absorption value of ca. 25% and for dilutions a highest value of 70% with only 4 values above 40% was obtained (Fig 1. and Table 1, and Fig. 2 of the CRD Report respectively).

For the 5 compounds with the highest dermal absorption values for the concentrate further investigations were performed to determine the type of data used to reach the conclusion (Table 3). It is considered that if well performed human *in vitro* studies had had been available on these products as concentrates, dermal absorption values lower than those derived by PRAPeR would probably have been obtained. A similar evaluation of the data for the 6 dilutions with the highest dermal absorption is presented in Table 4. In this case the picture is less clear as the two highest values do not appear to be markedly conservative.

Dermal absorption values for dilutions were nearly always greater than for the corresponding concentrates (Figs. 1 and 2 of the CRD Report).

An analysis of the highest values for both concentrate and dilutions showed that although many of them came from *in vivo* rat studies, human *in vitro* data and rat *in vivo* data corrected for rat-human ratios also produced relatively high dermal absorption values with some products (Tables 3 and 4).

Table 3: Details of active substances with the highest PRAPeR agreed dermal absorption values for the concentrate

Active substance	Formulation	Dermal absorption	Basis	Comment
Benthiavalicarb	15% WG[1]	16%	Human *in*	Includes all residue in skin sample

			vitro	(15%), no tape stripping.
Bifenthrin	10% EC[2]	18%	Rat *in vivo*	Short duration study, high skin residue - all included.
Myclobutanil	20% EW[3]	25%	Rat *in vivo*	Poor studies, no *in vitro* correction applied, high skin residue included.
Napropamid	45% SC[4]	26%	N/A	Poor studies, based on dilution value.
Quizalofop-P-ethyl & P-tefuryl	4% SC	18%	Rat *in vitro*	Includes all residue in skin sample including tape strips.

[1]Water dispersible granule, [2]Emulsifiable concentrate, [3]Emulsion, oil in water, [4]Suspension concentrate

Table 4: Details of active substances with the highest PRAPeR agreed dermal absorption values for the dilution

Active substance	**Formulation**	**Dermal absorption**	**Basis**	**Comment**
Acetochlor	84% EC[1]	50%	Human *in vitro*	48 hour study (8 h exposure), excludes tape strips
Benthiavalicarb	15% WG[2]	70%	Human *in vitro*	Includes all residue in skin sample (16%), no tape stripping but low recovery.
Bromuconazole	20% SC[3]	45%	Rat *in vivo*	*In vitro* correction not used due to poor quality.
Clethodim	20% EC	42%	Rat *in vivo*	Same value from 24h exposure and no skin residue or 10h exposure + skin residue.
Active substance	**Formulation**	**Dermal absorption**	**Basis**	**Comment**
Fenbuconazole	5% EW[4]	30%	Human *in vitro*	Supported by rat *in vivo* on an EC formulation. Based on peak flux x 24h; get value of ca. 20% based on receptor fluid + residue in stripped skin.
Fenoxaprop-P	6.8% EW	36%	Rat *in vivo* corrected	Human *in vitro* value higher than rat *in vitro* after 24h exposure; due to skin residue of 41% versus 11% respectively.

[1]Emulsifiable concentrate, [2]Water dispersible granule, [3]Suspension concentrate, [4]Emulsion, oil in water

It is concluded that the PRAPeR data for the concentrates indicate that a default dermal absorption value of 25% could be supported. For dilutions, a default of 75% is supported.

4.1.2. Analysis of dermal absorption values versus molecular weight, log Pow and dilution

For 63 of the active substances considered by PRAPeR an analysis of the agreed dermal absorption values (excluding default values) versus octanol/water partition coefficient (log Pow) was undertaken for both concentrate and dilutions (Annex 5 of the CRD Report). It is accepted that this analysis has flaws in that it combines results from several types of studies and with variable interpretations. In particular, it was noted that dermal depot was often taken into account when determining the dermal absorption values; this is a conservative approach and might confound the relationship between log Pow, MW and dermal absorption. The data indicate that for pesticide formulations (concentrates) evaluated at PRAPeR, log Pow is not a reliable predictor of dermal absorption based on the agreed dermal absorption values (Figs. 1 and 2 of the CRD Report). Low absorption (< 5%) appears to be associated with active substances with a negative log Pow but this group only includes 7 active substances which is considered being too small for drawing general conclusions. For dilutions there

appears to be a lower potential for dermal absorption when log Pow is outside the range of +2 to + 5, but there is no clear association or trend. It is also of note that log Pow can change with pH (Nielsen et al., 2009) and that pesticide formulations cover a range from pH < 3 to pH > 10.

An analysis of dermal absorption versus molecular weight was performed for both concentrate and dilution but provided no clear relationship (Figs. 3 and 4; Annex 5 of the CRD Report). Reasons for this could include the variations in study design and interpretation, and the fact that most pesticides have molecular weights within a limited range (100 - 500). In addition, pesticides of similar molecular weights can have markedly different chemical structures and sizes, and therefore the molecular weight might not be a good predictor of molecular size within this heterogeneous group of chemicals (Nielsen et al., 2009).

On the basis of theoretical considerations and results reported in literature (reviewed in de Heer et al., 1999) and in the absence of valid measured data, as a pragmatic approach for dermal absorption it is suggested to apply a lower default value of 10% for concentrate and dilution, for an active substance having a log Pow either lower than -1 or higher than 4 and a molecular weight of more than 500. This default value is in the current guidance on dermal absorption and data analysed above shows that there is no compelling reason to change this.

4.1.3. Analysis of human *in vitro* data versus PRAPeR agreed dermal absorption value based on corrected rat *in vivo* data.

An analysis of the outcome of the use of the "triple pack" approach was also performed. The analysed group consisted of 23 active substances/products. Results are summarised in Table 5. Where sufficient information was available, the human *in vitro* data were re-evaluated in a consistent manner in terms of the inclusion of the residue in the skin sample. The results show that in 9 out of 45 values, for 8 out of 23 compounds, human *in vitro* values were lower than the PRAPeR conclusion based on the triple pack approach (i.e. this approach gave more conservative values than human *in vitro* tests).

For the compounds where the agreed value was above the human *in vitro* value there were sometimes discrepancies that might have had an impact on the outcome. It is unclear from the available documentation whether or not these discrepancies were taken into account in the overall decision:

- Bromuconazole has poor reporting of the *in vitro* study but this is unlikely to result in the huge variation seen. Another bromuconazole formulation (emulsifieable concentrate) gave human *in vitro* dermal absorption values of 30 – 70%.

- Fuberidazole was assessed with an *in vivo* study performed under semi-occlusive conditions but the human *in vitro* study appears to have used dry material and unoccluded conditions, which could lead to low dermal absorption. This hypothesis is further supported by the low absorption of the diluted versus the concentrated material.

- Lufenuron had no obvious discrepancies in terms of inclusion of application site/tape strip residues. However the values for the receptor fluid results with human skin were reported to be at the limit of quantification (LoQ) which could result in a falsely high value taken into the human:rat correction. The rat:human correction for the dilution was 1:1 whereas that of the concentrate was 6.5:1.

- Metamitron had the *in vitro* correction based on % absorbed (2.5:1) rather than flux (5.1:1). Had a 5.1:1 correction been used, the corrected value would have been below human *in vitro* value. In any case the difference is trivial.

- Myclobutanil had the *in vivo* study performed with an emulsifiable concentrate formulation but the human *in vitro* study used an oil in water formulation. Also the higher absorption of the concentrated versus diluted formulation should be noted.

- Tebufenozide had the *in vivo* study performed with 10 or 24 hour exposures and no removal of the compound prior to the end of the study. *In vitro* there was a 6 hour exposure with sampling for a further 18 hours. The formulation tested *in vivo* has a different code to that tested *in vitro* and the relationship between the two products is unknown.

- Triazoxide had rat and human *in vitro* values below the corresponding rat *in vivo* results but there were no obvious discrepancies between the *in vivo* and *in vitro* studies. Dermal absorption for the concentrate based on the human *in vitro* data appears to be below the PRAPeR agreed value.

- Triflumuron had the agreed value for the concentrate rounded up to 1%. Without rounding the value would have been circa 0.1%, which is below the human *in vitro* value. For the dilution the amount in the skin sample *in vitro* represented the vast majority of the test material considered to be absorbed for both rat and human samples even though the study was run for 72 hours. Whereas for the *in vivo* study the skin residue represented < 1% compared with circa 14% that was in the carcass or excreted. The reason for this difference between the pattern of *in vitro* and *in vivo* results is unknown.

To sum up, for 4 compounds data were not reliable: in one case it was an issue related to different rounding criteria, in one case flux had not been used for correction, in one case the human *in vitro* results were very low and appear to have been influenced by the limit of quantification (LoQ). In addition in two cases there was no obvious reason why the agreed values based on the triple pack approach gave a result that was above the value obtained from human *in vitro* data alone. Without corroboration with human volunteer data it is not possible to determine which method gives the most appropriate value in these cases.

The PPR Panel concluded that taking account of the variations in study performance and interpretation, human *in vitro* data can be used as stand alone data to predict dermal absorption for dermal exposures to pesticide products as both concentrates and dilutions, and that the triple pack approach may not necessarily lead to a significant refinement of the values.

Table 5: Comparison of human *in vitro* dermal absorption with values obtained from rat *in vivo* and rat and human *in vitro* data (n=23)

Compound	Triple pack approach		Dermal absorption (%), human *in vitro*	
	Concentrate	Dilution	Concentrate	Dilution
Acetochlor[1]	0.5	4	0.5	6
Benfluralin	0.6	4.5	2.6	10
Bromuconazole	5	45	7	***1***
Cymoxanil	1	5	No mass balance	26
Cyromazine	0.7	2	9	8
Dimethachlor	3	10	7	28
Dodemorph	2.7	20	4	28
Fenpropidin	2.5	6	49	48
Fenoxaprop-P-ethyl	1.6	36	18	59
Fluazinam	1.5	7	4	47
Fludioxonil	0.3	1.7	79	3
Fluopicolide	0.2	2.8	0.2	3.6

Compound	Triple pack approach		Dermal absorption (%), human *in vitro*	
Fluquinconazole	1	4	4	16
Fuberidazole	1	6	1	***0.4***
Lufenuron	2	13	3	***5***
Metamitron	1	20	***0.8***	21
Myclobutanil	25	15	***6***	24
Penconazole	1	5	20	50
Tebufenozide	0.2	2	0.2	***1.3***
Teflubenzuron	2	20	2	25
Tetraconazole	1	20	2	37
Triazoxide	2.4	2	***0.7***	2
Triflumuron	1	5	***0.2***	***3***

[1]Tested in form of a capsule suspension

4.2. CRD database: comparison of dermal absorption with formulation type.

A total of 340 dermal absorption values were available from the CRD database which is available from https://secure.pesticides.gov.uk/TEAWeb/search.asp (see also Annex 6 of the CRD Report) and which includes all the values agreed by PRAPeR. An evaluation of active substances having dermal absorption data on more than one formulation type was performed, but it was too limited to permit any meaningful comparisons (see Annex 7 of the CRD Report). Therefore, no general conclusions could be drawn from this database on the impact of formulation type on dermal absorption.

4.3. EDETOX database

The EDETOX project was a multicentre project funded under the 5th EU Framework Programme to investigate various aspects of dermal absorption and its use in risk assessments of human exposure to chemicals. It is available from: http://research.ncl.ac.uk/edetox/index.html. Amongst its conclusions was one relating to the need for QSAR models to take account of vehicle effects and another supporting the usefulness of *in vitro* data. Many of the detailed investigations performed during the EDETOX project used only a small number of compounds (n < 5) which limits the general applicability of some of the conclusions.

A major output of the project was a database of published literature on *in vitro* and *in vivo* dermal absorption. The database contains an extensive compilation of data in a searchable format. It contains 1657 *in vitro* data points (537 with human skin samples) and 844 *in vivo* entries (265 relate to human exposures). Notably there is some repetetive information due to multiple publications by authors. The highest dermal absorption value from an *in vivo* human study using a concentration of >1 g/L was 27% (2-phenylphenol, 40 g/L in ethanol) while for low concentrations (4mg/L) dermal absorption in humans *in vivo* was as high as 74% (carbaryl in acetone).

4.4. Conclusions from existing data and proposal for default values

The existing dermal absorption data on plant protection products are very variable in terms of study design, data reporting, summarisation and interpretation.

Based on the evidence evaluated, and within the limitations described, the following conclusions were reached:

- Literature references report that dermal absorption of a chemical is affected by a range of parameters including irritancy, presence of solvents and surfactants, site of application, *in vitro* sample thickness and application site loading.

- Data generated using plant protection products showed that:

- dermal absorption as a proportion of the applied dose is generally higher from diluted than concentrated products.
- there is no clear link between formulation type and dermal absorption.
- there is no clear link between dermal absorption and either octanol/water partition coefficient (log Pow) or molecular weight.

- Dermal absorption from plant protection products should therefore be determined on the formulation to be sold and representative dilution(s) thereof.
- Data generated using plant protection products and other chemicals showed that for concentrated products dermal absorption is unlikely to exceed 25% and for dilutions of products it is unlikely to exceed 75%. Since the dataset of 63 active substances covers a wide range of pesticide formulations it is the opinion of the PPR Panel that the 5% concentration can be used to define diluted (≤ 5%) and concentrated (> 5%) products to which the 75% and 25% absorption values can be applied, respectively. In fact, as reported in sections 4.1.1. and 4.3. dermal absorption of > 27% is only seen with an active substance content < 4%.
- The existing default of 100% in the current Guidance Document (EC, 2004) needs to be revised.
- There is no compelling reason to modify the existing default of 10% for substances with log Pow < -1 or > 4 and MW > 500 (EC, 2004).
- Analysis of the results of the "triple pack" approach and of human *in vitro* data, indicates that the latter can be used as a stand alone test to derive dermal absorption values in humans.

5. Other considerations

5.1. Use of oral absorption data

It has sometimes been argued that dermal absorption cannot exceed the oral absorption rate. Accordingly it could be possible to obtain an indication of the dermal absorption of a compound from ADME data providing information on oral absorption. In the absence of specific experimental data, dermal absorption of a certain substance can be assumed to be at maximum the same percentage as that established for the oral absorption rate in an ADME study. There are not enough data to scientifically validate this assumption except for unpublished data on direct comparison for 12 pesticides quoted in the current Guidance Document (EC, 2004). However, based on practical experience, it is very likely that it holds true for most substances in spite of the considerable differences of the absorption mechanisms from the gut and through the skin. Exceptions may apply to substances with very poor oral absorption especially taking into account that there are usually no oral ADME studies for formulations that include co-formulants which are possibly modifying dermal absorption. For these reasons, estimates based on oral absorption should be applicable in only a limited range of circumstances after careful consideration of doses and vehicle used in the ADME studies, where bile-cannulation was also performed.

5.2. Use of dermal and oral toxicity data

It can be possible to obtain an indication of the dermal absorption of a compound by comparing the toxicity produced at different dose levels via the oral and dermal routes.

Since there are significant shortcomings associated with this approach, it is not recommended and should only be applicable in a limited range of circumstances. While acute toxicity studies should not be used under any circumstances, a number of conditions should occur in order to allow such an approach with repeated dosing studies; these may include:

- The oral and dermal toxicity studies are performed in animals of the same strain and sex.
- The material tested is closely related to the product under evaluation.
- Study duration and end-points investigated are comparable.
- The toxicity profile in terms of endpoints (death or any other effect deriving from a single dose is not an appropriate endpoint) and timing is similar by both routes and is associated with the active substance not a co-formulant.
- Lowest Observed Adverse Effect Levels (LOAELs) and No Observed Adverse Effect Levels (NOAELs) are available from both dermal and oral routes, unless the highest dose tested in the dermal study is a NOAEL.
- First pass metabolism is not extensive.
- Dermal absorption is not saturated due to a high loading of material at the application site (i.e. application rate is not above 10µl/cm^2). This is unlikely to be the case with limit dose tests.

If these criteria are met, an indication of the dermal absorption can be obtained by comparing the LOAELs from both dermal and oral routes, unless the magnitude of the response at the dermal LOAEL is clearly greater than at the oral LOAEL, in which case the dermal NOAEL should be used.

For example:

oral LOAEL: 10 mg/kg bw/d
oral NOAEL: 5 mg/kg bw/d
dermal LOAEL: 100 mg/kg bw/d
dermal NOAEL: 50 mg/kg bw/d

For a similar response: oral LOAEL (10 mg)/dermal LOAEL (100 mg) x 100%: the dermal absorption is likely to be around 10%.

For greater response at the dermal LOAEL: oral LOAEL (10 mg)/dermal NOAEL (50 mg) x 100%: the dermal absorption should be taken as 20%.

5.3. Rounding of values

Usually studies have a relatively high variability. Therefore, values are rounded to 2 (≥ 10%) or 1 significant figures (1 to < 10%). For absorption values < 1%, there is no reason to round to 1% if data are consistently showing values < 1%. For example, for the fungicide azoxystrobin the dermal absorption from a rat *in vivo* study was 0.3% including application site residue, from a rat *in vitro* study it was 1.2% including skin residue and from a human *in vitro* study ca 0.2%. The consistency of the data indicate that dermal absorption is likely to be significantly less than 1% and rounding up to 1% is not justified. Rounding should be applied only to final values and not to intermediate values obtained during the calculations.

5.4. "Triple Pack" approach

It might be possible to use data from a rat *in vivo* study corrected for the ratio of absorption in rat and human skin *in vitro* ("triple pack" approach). This approach has the advantage that the *in vivo* study utilises viable skin and can be run for an extended period (e.g. 7 days or longer) to permit a more detailed investigation of the absorption time scale. In order for the approach to be scientifically valid it is essential that the study protocols *in vitro* are well matched for variables that could influence the results e.g.:

- Skin type (i.e. split-thickness)
- Test material/formulation/vehicle
- Exposure duration and sampling period
- Receptor fluid composition
- Swabbing technique
- Analytical techniques

The *in vivo* study should use the same test material/formulation/vehicle, area dose (concentration), and a similar exposure time and swabbing technique as the *in vitro* studies.

Normally this will be achieved by performing the studies contemporaneously in the same test facility, however, this is not an essential requirement. If the *in vitro* studies are not well matched then it is either not valid to perform a comparison of the relative dermal absorptions or it should be done with caution.

If the *in vitro* studies are closely matched then the relative absorption can be estimated by taking the ratios of either the maximum flux or % absorption. The maximum flux should normally be calculated from a linear portion, of 2 hours or longer, of the absorption time curve. A shorter time period may be used if absorption is very rapid and essentially complete within 4 hours. There are debates about whether the maximal flux or percentage absorbed is the best method to determine the relative absorption of rats versus humans. Either option can be taken since there are no strong arguments in favour of one or the other.

There are circumstances when the flux might not be appropriate e.g. the linear phase is significantly longer in the human samples. In these cases the comparison can be performed using the % absorbed but it must have the same basis for both rat and human samples i.e. in terms of inclusion of tape strip material or residue in the skin sample.

5.5. Read-across and (Q)SAR

(Q)SAR studies have addressed the issue of structure permeability relationships, or other approaches, including read-across, that try to derive absorption parameters based on chemical structure and other physico-chemical parameters. These are discussed in WHO (2006) and Bouwman et al. (2008). However, for pesticide formulations, where the influence of other determinants is relevant, as described above, such methods do not find proper application, although such an approach might be feasible in some certain instances.

5.6. Microencapsulated formulations

Limited data are available on the dermal absorption of active substances from microencapsulated formulations. In principle, absorption from microencapsulated formulations is lower than that of the non-encapsulated product, but general rules cannot be provided. Another issue that needs to be considered is whether exposure would be to the microencapsulated product or to the product within the capsules. In order to use dermal absorption values determined for the microencapsulated product, data should be provided on the resilience of the capsules to the procedures used in application of the product or once it is in contact with the skin. This is a less relevant issue for exposure of re-entry workers and residents. They are most likely exposed to the product within the capsule, since it seems reasonable to assume that it has to be released in order to be effective.

5.7. Nanoformulations

Although so far there are no registered pesticides on the market which contain nanomaterials, progress of science indicates that this might be the case in the future, in particular since there are already biocides with these characteristics. Regarding the assessment of risks in nanotechnology, the Commission's independent Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has already published several opinions (SCENIHR, 2006; 2007; 2009). The latest one, published in January 2009, indicates that methodologies to assess exposure to manufactured nanomaterials and the identification of potential hazards to humans and the environment require further development, that more research is needed and that risk assessment should be performed on a case by case basis for each nanomaterial.

Also EFSA's Scientific Committee has already published a scientific opinion on nanoscience and nanotechnologies in relation to food and feed safety (EFSA, 2009b). The Scientific Committee concluded that established international approaches to risk assessment can also be applied to engineered nanomaterials (ENM), but that a case-by-case approach would be necessary and that, in practice, current data limitations and a lack of validated test methodologies could make risk assessment of specific nanoproducts very difficult and subject to a high degree of uncertainty.

In particular, a recent review on dermal absorption and toxicity of nanoparticles (Crosera et al., 2009) indicated that there are few studies on skin penetration and that results were inconsistent because of differences in methods, laboratory conditions and absence of standardised evaluation protocols. It was underlined that characterisation of nanoparticles is essential with respect to for instance size, shape, coating, purity, presence of catalyst, extent of agglomeration and agglutination. Consequently, at present no guidance can be given on how to assess and evaluate dermal absorption of such materials.

5.8. Re-entry workers

The choice of the dermal absorption value to use for re-entry workers and residents who are exposed to a dried dispersed residue is a controversial area. Theoretically there are two approaches that can be justified. The material is dry and therefore absorption could be considered to be related to that of the concentrated product. However, the loading of active substance per unit area is more closely related to that of the spray dilution. In reality, the most appropriate approach might be unrelated to the absorption characteristics of either the concentrate or spray dilution. Research is being performed to investigate this issue (DEFRA, 2009). As in interim measure we suggest using the highest dermal absorption value identified.

5.9. Use of data from field studies

Results from field studies, if well conducted, and especially biomonitoring data, may be helpful to confirm results obtained from experimental dermal *in vivo* and *in vitro* testing. Moreover, a field study carried out with a substance that is chemically related to the substance of interest, with similar

physicochemical properties, may also be taken into account. It should be noted, however, that in such studies an accurate measurement of skin deposition is almost impossible to perform and the metabolism in humans needs to be well known both qualitatively and quantitatively. Therefore estimated absorption is rarely accurate, but data can be used to support values experimentally determined.

6. Proposed guidance

A guidance based on the considerations presented has been produced as a separate document. We believe that application of this guidance would result in a more harmonised approach to dermal absorption in EU risk assessment of PPPs. In particular, the guidance that is aimed at replacing the existing guidance document provides more detailed and reasoned indications on how to proceed.

The new guidance will be finalised and published only after adoption and publication of this opinion.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The PPR Panel concludes that, in the absence of specific studies assessment of dermal absorption can be performed based on default values that the Panel derived from the analysis of the available databases. Depending on availability of studies, elements for a tiered approach will be presented in the guidance, which will be finalised after adoption of this opinion. The PPR Panel concluded that the *in vitro* human study can be used as a stand alone test to derive dermal absorption values for risk assessment, thus not encouraging studies on animals when scientific valid alternatives are available.

The PPR Panel notes that there are no good predictors of absorption from pesticide formulations and therefore the PPR Panel is of the opinion that dermal absorption data on plant protection products should be generated on the formulated product, or a closely related product, and on concentrations representative of the spray dilutions as applied to the crop.

Available data were considered inadequate to draw any conclusion regarding the potential to extrapolate between formulation types; in this case use of default values is suggested.

The Panel identified that skin irritation enhances dermal absorption. Also other factors influence dermal absorption of chemicals, including octanol/water partition coefficient, molecular weight/size, use of detergents/solvents and the formulation type, albeit, in a way which cannot generally be predicted. However, dilution of the active substance generally increases the dermal absorption.

The PPR Panel concludes that the possible presence of skin damage to a limited skin area, or skin diseases such as atopic dermatitis, will not significantly impact on dermal absorption of pesticide formulations in normal use and that they are covered by the safety factors applied when deriving the acceptable operator exposure level (AOEL). Also minor differences in skin absorption due to age do not necessitate the establishment of different absorption values for children and adults.

Since according to EU Regulation (EC) No 1107/2009 data from human volunteer studies cannot be used to perform risk assessment, only issues related to studies in non-human primates are discussed although most of the considerations will also apply to studies in humans. There are difficulties in such studies relating to the determination of the overall mass balance. The PPR Panel is of the opinion that in any case, experiments with non-human primates are difficult to carry out and to be interpreted. Therefore, their use is discouraged, and the recommendations provided in the guidance solely refer to already existing studies.

The Panel also identified the need for a harmonised reporting of the studies that will help in taking decisions that are consistent across time and EU member states.

In summary, the approaches recommended in this opinion are based on a combination of methods and default values. Some of these are based on evaluation of data but others are based on recommendations in other regulations/guidance for which the Panel could not find clear scientific support. Overall, taking account of the uncertainties involved, it is the Panels opinion that the dermal absorption estimate will be sufficiently protective.

RECOMMENDATIONS

The PPR Panel recommends that a new guidance document on dermal absorption should be adopted along the lines of that are set out in a separate document.

The guidance should be reviewed periodically and if appropriate revised as well as when relevant new data becomes available.

Further research could usefully be conducted on:

- default values
- QSAR and read-across
- skin lesions and diseases (relevance and occurrence)
- evaluation of the proper experimental conditions to mimic use of personal protective equipment (PPE)
- extrapolation between formulation types
- identification of the most appropriate approach to define dermal absorption values for re-entry workers (diluted vs. concentrate)
- studies on dermal absorption of nanoformulations

DOCUMENTATION PROVIDED TO EFSA

1. Guidance Document on Dermal Absorption (Sanco/222/2000 rev. 7).

2. Draft Guidance Notes for the Estimation of Dermal Absorption Values (OECD).

3. Draft data requirements Revision of Annexes II and III to Directive 91/414/EEC (Sanco/10482/2006 rev.11).

4. Guidance Document for the Conduct of Skin Absorption Studies (OECD Environmental Health and Safety Publications, Series on Testing and Assessment No. 28, 2004).

5. Dermal Absorption (Environmental Health Criteria 235, International Programme on Chemical Safety (IPCS), 2006).

6. OECD (2004a) Guideline for the Testing of Chemicals 427 Skin Absorption: *in vivo* Method

7. OECD (2004b) Guideline for the Testing of Chemicals 428 Skin Absorption: *in vitro* Method

8. Opinion of the Scientific Committee on Plants on Commission Draft Guidance on Dermal Absorption (Doc. SANCO/222/2000-rev.4 dated 11 April 2001; adopted by the Scientific Committee on Plants 24 April 2002).

9. Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of dinocap in the context of Council Directive 91/414/EEC, *The EFSA Journal* (2004) 74, 1-23.

10. Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of methamidophos in toxicology in the context of Council Directive 91/414/EEC, *The EFSA Journal* (2004) 95, 1-15.

11. Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on the scientific principles in the assessment and guidance provided in the field of human toxicology between 2003 and 2006, *The EFSA Journal* (2006) 346, 1-13.

12. Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the revision of Annexes II an III to Commission Directive 91/414/EEC , *The EFSA Journal* (2007) 449, 1-60.

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APPENDIX

A. RESPONSES TO THE COMMENTS RECEIVED IN THE PUBLIC CONSULTATION ON THE CURRENT GUIDANCE DOCUMENT ON DERMAL ABSORPTION (SANCO/222/2000 REV. 7, 19 MARCH 2004)

Responses to specific comments are given with the indication of the relevant paragraph in the Opinion (OP) and/or revised Guidance (GD), whereas responses to general comments give only an indication of how and if these have been addressed. Responses are in *italic*.

No	Institution	Chapter	Stakeholder comment and PPR Panel response
1	Institute of Public Health, Slovenia	0. General comments	The guidelines should be optimised in order to give fewer options for choice to the notifier and consequently allow better comparability of data. This would also allow the creation of a database of comparable results which should give essential information for further developing of the second tier evaluation (cut/off criteria) in the future. It will also facilitate read-across approach. *Efforts have been made in order to provide guidance for a consistent and harmonised choice of options.* *The creation of a database is outside the scope of the GD.* Where expert judgement is mentioned examples would be very useful. *Whenever possible examples or detailed procedures have been described. Flow charts are presented to facilitate the assessment.*
2	Federal Office of Consumer Protection and Food Safety, Germany	0. General comments	The guidance document on dermal absorption has proven to be very useful. However, some revisions and amendments are in fact needed. Because the Federal Institute for Risk Assessment (BfR) will actively participate in the next stages of the revision process and will certainly provide much more detailed comments, the focus of this contribution is on identification of those parts of the document that should be substantially amended and of additional issues that are not addressed so far but should be included in the revised version. General comments: The possible sources of information on dermal absorption should be mentioned, per-haps in a summary table, which can be used instead of the (unrealistic) assumption of a 100 % default value. The advantages and limitations of the different methods and approaches and their very different reliability for prediction of the dermal absorption rate should be highlighted.

No	Institution	Chapter	Stakeholder comment and PPR Panel response
			New default values have been provided, with justification. *A discussion of the different methods has also been included.* A clear recommendation should be given to use experimental data obtained in valid dermal absorption studies if available because of their much higher reliability as compared to, e.g., the comparison between oral and dermal short-term toxicity studies or the various non-experimental methods (consideration of physico-chemical properties, QSARs, "Read-across" etc.). All the latter information, at best, can be accepted to provide rough estimates of dermal absorption that will allow refinement (i.e., reduction) of the default value to rounded figures such as 50 %, 25 %, 10 %, 5 % or 1 %. *These points have been addressed and flow charts are presented to facilitate the assessment.* Even results from properly conducted dermal absorption studies should be considered with care and some reservation. In any case, results should be preferably rounded to whole numbers. It might be recommended to use 1 % as a "worst case" for substances for which "very low" dermal absorption has been experimentally proven. *Proposal for criteria accepting less than 1% absorption has been included.* Formulations of active compounds are authorised by the MS but not the actives themselves. Naturally, for technical and ethical reasons and because resources are limited, it is not possible to test all formulations. Accordingly, guidance should be given under which conditions data from one formulation can be applied to another containing the same active ingredient(s). i.e., how to define a "similar" formulation or how to define significant differences. For this purpose, the possible impact of co-formulants, mixtures of compounds and dilution factors must be addressed. Guidance should also be given regarding the transfer of absorption data based on the active substance alone. Gaps in our knowledge should be clearly stated as such. *Guidance on these aspects has been provided.* References should be checked to find out whether in fact the best and most reliable of the available scientific information has been included. *References have been checked.*

No	Institution	Chapter	Stakeholder comment and PPR Panel response
3	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	0. General comments	It is essential that the next version of Sanco/222/2000 does not conflict with the OECD Guidance notes for the estimation of dermal absorption values (the draft of 26 May 08 is now being revised by an expert group lead by Australia). Collaboration between the two activities should therefore be initiated. *Agree where feasible, but OECD remit has been expanded to cover chemicals in general. Frequent contacts with OECD have been kept.*
4	Swedish Chemicals Agency (KemI)	0. General comments	We are concerned about testing of chemicals on humans, particularly pesticides, as these chemicals are toxic per definition. However, if such dermal absorption studies are already available, they should be used only if they show a higher dermal absorption than already obtained in the animal studies. *Since the use of human data is not allowed in EU, neither the ethical nor the scientific issues related to their use have been discussed.*
5	Charles River Laboratories, UK	0. General comments	This is a general comment that in sections 4.2 and 4.4 there is no mention of stratum corneum for calculation of results (only "skin" mentioned). Therefore, especially for *in vitro* studies, there should be further clarification on if, when referring the "skin" this document means: 1. viable skin (epidermis and dermis) + all stratum corneum. 2. viable skin (epidermis and dermis) - all stratum corneum. 3. viable skin (epidermis and dermis) + lower layers of stratum corneum. If (3.) is the most accurate description then further clarification is needed on what constitutes "lower layer" (i.e. how many tape strips). If this is dependant upon results (flux data etc) then this should also be clarified. Whilst I have focused on *in vitro*, I believe this may also be applicable for *in vivo* studies where there are no serial non-detects in urine or faeces. One other minor point is OECD references need updated to 2004 in section 3.1. *All these issues have been addressed and guidance has been provided.*

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6	Charles River Laboratories, UK	0. General comments	General suggestions: There is very little discussion on the issue of the stratum corneum. There is no discussion on Ficks 2nd Law of diffusion. Since absorption is Ficks 2nd Law of diffusion, it is important to define what is the semi-permeable membrane. It is generally accepted that the membrane is actually the stratum corneum. This makes it essential to include the "viable" skin, but not the stratum corneum in the risk assessment (in line with the citation for EPA, 1992). However, it does not take into account the potential stratum corneum reservoir. With stratum corneum profiling detailed, it is much simpler to decide if all/ some/ none of the stratum corneum should be included in the risk assessment especially if compared with the absorption profile. Should you require any further comment or require my input in person, then I will be available. *All these issues are addressed in detail in the GD and in the OP.*
7	Dow Agrosciences, UK on behalf of the European Crop Protection Association (ECPA), Belgium	0. General comments	Unfortunately, due to time constraints, ECPA Members* were unable to provide comments on all of the concerns held with the existing guidance document. In addition, some detail has been lost due to the limitations set by EFSA on the length of individual comments. ECPA Members hope to be able to rectify these omissions during future steps of the review process. ECPA Members believe that the critical points that require revision include: • New guidance on default absorption values based on database of studies conducted in accordance with OECD 427 and 428 • New guidance on tape stripping to cover both *in vitro* and *in vivo* studies • New guidance on the use of read across between formulation type and/or related active substances • Revision on guidance for the definition of end of *in vivo* absorption *EFSA note: Individual names are not disclosed. *All these issues are addressed in the GD.*
8	Technology Sciences (Europe) Limited (TSGE), UK	0. General comments	Discussion of the general principles of read-across between tested formulations may be useful. This may include the following points: It may be possible to extrapolate a dermal absorption value generated using a solvent-based formulation to a water-based or solid formulation as dermal absorption from the solvent-based formulation is likely to represent a worst case. Similarly it may be possible to extrapolate from a water-based formulation to a solid formulation. However extrapolation in the other direction is not possible.

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			This extrapolation was investigated but found to be generally not feasible. The criteria when it is feasible to read across between formulations are given in the GD. It may be possible to extrapolate a dermal absorption value generated using a product dilution to the product concentrate, as this is likely to be a worst case. *Guidance is provided in this respect.* Similarly it may be possible to extrapolate a dermal absorption value from a more dilute product to a less dilute product, however extrapolation from a less dilute product to a more dilute product should be addressed on a case by case basis and is unlikely to be acceptable for a significant difference in concentration (e.g. 2-3 times?) unless existing data indicate that absorption is not strongly influenced by concentration. *Guidance is provided in this respect.*
9	Technology Sciences (Europe) Limited (TSGE), UK	0. General comments	Inclusion of a discussion of tape-stripping is needed. This should include its potential role in demonstrating the likely systemic availability or non-availability of the skin residue and the identification of surface residue (1st 2 tape strips). *Guidance is provided in this respect.*
10	Institute of Public Health, Slovenia	1. Introduction	The second paragraph (indented) should preferably be moved out from the Introduction into a separate chapter, maybe placed before the Introduction. Maybe it should be rewritten to clearly separate what is the rule and what are exceptions in specific situations. *The introduction has been re-written.*
11	Federal Office of Consumer Protection and Food Safety, Germany	1. Introduction	The limitation of the scope of the document to the 3d stage of the evaluation programme must be deleted. *The introduction has been re-written.* It should not be allowed to use 10 % default value for ongoing evaluation without being justified by physico-chemical properties etc. *New proposals on default values are given, with justification.* In addition, the revised version should be placed properly into the context of other regulatory documents on dermal

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			absorption such as the OECD guidelines 427 and 428, the OECD guidance document, or the comprehensive IPCS (EHC) paper on dermal absorption. The "OECD guidance notes" on harmonisation of study interpretation and the approaches to be taken if there is no specific data available, which are currently being drafted by an expert group, should also be taken into consideration. *See comment 3.*
12	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	1. Introduction	Page 2, Introduction, first paragraph: It should be more explicitly stated that this Guidance Document is used for the risk assessment of operators, workers and bystanders and that the whole document is applicable to these groups. *The point is covered in the new introduction.* Page 2, third paragraph: Some exposure models for the operator are mentioned, but not for the bystander and worker. Since this GD does not cover exposure models, it is advised to remove these models from the text. *Agree.*
13	Swedish Chemicals Agency (KemI)	1. Introduction	It should be emphasized in the introduction that for product authorisation, dermal absorption studies on the product are required. If studies are not available on the product applied for, studies on related products can be used if the chemical compositions of the two are similar. A suggestion is to list formulants that should be paid special attention to, e.g. ethanol (as in Appendix III of the OECD draft document Guidance Notes for the Estimation of Dermal Absorption Values, 26 May, 2008). *Guidance is provided in this respect.* *A list of solvents of concern has not been produced.*
14	Charles River Laboratories, UK	1. Introduction	Paragraph 3, last line: OECD, 2004a, b, c (draft 2000 replaced by final 2004). This needs to be checked throughout the entire document. *References are updated*.
15	Dow Agrosciences,	1. Introduction	Comment 1: The following text "To provide a reliable framework … when applying the Uniform principles." should be replaced by: "Since the release of the initial Guidance Document (Rev.7), dermal absorption studies conducted in accordance with

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	UK on behalf of the European Crop Protection Association (ECPA), Belgium		OECD 427 and 428 test guidelines have been conducted and submitted as part of the EU review process. Therefore, now it is possible to determine realistic default dermal absorption values based on this extensive empirical database, which covers the impact of formulation type on absorption of an active substances from a PPP and its spray dilutions." This revision is required because the use of physical-chemical properties of an active substance has been shown to be an inadequate predictor of dermal absorption of an active substance from a formulation and its spray dilutions. This can be demonstrated by a comparison of the physical-chemical properties and dermal absorption values of an active substance published as CEP's in the EU review process and therefore this comparison should be a part of the EFSA review procedure for this Guidance Document. Comment 2: The following text "This document provides … guidance on how to conduct relevant dermal absorption studies" should be deleted. *Introduction has been re-written.* This revision is required because guidance on how to conduct studies is contained in the OECD test guidelines (427 and 428) and also in the specific OECD Guidance Document (#28) for conduct of these studies. Additional guidance on conduct is not required, or provided, in this guidance document. *Some suggestions, relevant for testing pesticides, are included in the GD.*
16	Scientific Institute of Public Health, Belgium	2. An overview of dermal absorption 2.1. Introduction	Line 12 up to line 24 (...1993): we propose a modification of the paragraph as following: "upon contact with the skin, a compound penetrates the outer layer, the stratum corneum (keratinocytes), which is basically impermeable to water and hence to water soluble polar chemicals. The stratum corneum also prevents evaporation of water from the underlying cell layers. Since in normal conditions the stratum corneum is highly hydrated, the skin can still take up polar substances, albeit slowly, through passive diffusion as governed by Fick's First law. The hair follicle shafts are thought to provide a further route of entry. This route is considered to be most important during early stages of absorption process, especially for lipophilic compounds. The sweat and sebaceous glands are also thought to confer permeation routes. Skin permeability is enhanced by hydration of the skin. Other factors affect penetrability such as changes in ambient temperature, damage to the horny layer as well as edema, solar irradiance, exposure to organic solvents. The second phase is diffusion of the chemical through the lower layers of epidermis and dermis. These cell layers are

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			far inferior to the stratum corneum as barriers. They contain porous, nonselective aqueous diffusion medium and chemicals pass through this area also by diffusion. The chemicals diffuse to the dermis where a microcapillary bed of blood vessels serves to carry the molecules away from the dermis. Once the permeating molecules have entered the blood, they are considered to be bioavailable. These different aspects explain that skin structure differs from one species to another." *The introduction has been rewritten with references to specific publications.*
17	French Food Safety Agency, AFSSA	2. An overview of dermal absorption 2.1. Introduction	The following documents/references could be mentioned in this introduction: OECD, Guidance notes for the estimation of dermal absorption values, draft may 2008WHO, IPCS/EHS 235, Dermal absorption, 2006. *Done.*
18	Charles River Laboratories, UK	2. An overview of dermal absorption 2.1. Introduction	Paragraph 2, last sentence: The stratum corneum is the barrier to absorption. As stated above, once in the viable epidermis it is in the living tissue. The Flynn reference should be deleted and replaced with something more suitable and far less abstract in thinking. *Deleted.* Paragraph 3, line 4. Suggest adding SCCP Guidance document (cosmetic products), although this is under review currently. *SCCS Guidance document on in vitro assessment of dermal absorption has been considered.*
19	Institute of Public Health, Slovenia	2. An overview of dermal absorption 2.2. The studied tissue	Three types of skin membranes can be used for *in vitro* experiments although for two of them disadvantages are mentioned in the same paragraph. I would prefer to see the split-thickness skin as the main choice and any deviations should be justified. That would reduce variability in testing which is important in creating a good database. *The use of split thickness skin is recommended in the new GD.*
20	Scientific Institute of Public Health, Belgium	2. An overview of dermal absorption 2.2. The studied tissue	If the previous comments are taken into account then the first 6 lines of point 2.2 could be deleted. The paragraph could start with "In case of *in vitro* studies..." *Introduction has been rewritten.*

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21	Estonian Research Institute of Agriculture, Estonia	2. An overview of dermal absorption 2.2. The studied tissue	2.2.- page 5, line 1. Scott(1991) - skin permeability could be related to species differences in skin structure..... -skin structure is depending on age of rats and maybe on strains of rats ? How to avoid this differences and mistakes? *In experiments with rats dermal absorption is over estimated in comparison to the human situation. Therefore strain differences are not addressed. Emphasis is rather given to in vitro studies with human skin.*
22	Charles River Laboratories, UK	2. An overview of dermal absorption 2.2. The studied tissue	Paragraph 1, line 10: full thickness skin is not recommended cf OECD TG 428. *Agree: split thickness skin is recommended.* The lower dermis will act as a significant sink, so partitioning to the receptor fluid and thus prediction of systemic availability will be far lower. This does not impact on the overall risk assessment because the "viable" skin should always be included as well. *Agree. This issue is commented on in the GD.*
23	Technology Sciences (Europe) Limited (TSGE), UK	2. An overview of dermal absorption 2.2. The studied tissue	Lines 14-15: *"epidermal membranes are more fragile and sometimes overestimate human in vivo skin absorption"* Some clarification of this statement is required: It is generally accepted that *in vitro* studies overestimate dermal absorption in human skin *in vivo* - this is the basis for the use of *in vitro* data outlined later in the document. However the statement above indicates that damaged membranes may be used, which is not the case if appropriate measurements of integrity are made. *The issue is addressed in the GD.*
24	Technology Sciences (Europe) Limited (TSGE), UK	2. An overview of dermal absorption 2.2. The studied tissue	Line 1 page 5 should read *"Scott et al (1991)"*. *The reference is deleted.*

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25	Scientific Institute of Public Health, Belgium	2. An overview of dermal absorption 2.3. Active substance properties	Point 2.3 line 1-11: We propose to include in the text somewhat more details such as: "Two intrinsic factors contribute to the absorption rate of a given compound: -hydrophobicity: this parameter is measured by the octanol/water partition coefficient (Kow) partitioning of an agent into the skin is greatly affected by its solubility in or adhesion to the medium in which it is applied. the physicochemical properties of the vehicle are very important in influencing the rate of percutaneous absorption, since they will regulate the vehicle/stratum corneum partition coefficient. -rate of diffusion through this barrier: this property is an inverse function of molecular weight or more accurately, of molecular volume. Ionic state plays an important role as non ionized molecules penetrate easily. however, substances which are in the ionized state at pH 7.4 may have a higher permetation coefficient than would be expected from Poct or by molecular weight." *These issues are addressed in the new GD.*
26	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	2. An overview of dermal absorption 2.3. Active substance properties affecting penetration	Please include "*penetration*" after the first parenthesis after the first bullet, otherwise the impression is created that liposolubility is at its maximum between log Pow +1 and +2. *Text has been rewritten.*
27	Technology Sciences (Europe) Limited (TSGE), UK	2. An overview of dermal absorption 2.4. Experimental conditions	Line 6: For compounds binding to the skin, dermal penetration at low concentrations is actually lower than at high concentrations. Similarly ionisation of compounds may increase with dilution, resulting in lower dermal absorption. *Guidance is provided on how to extrapolate between different dilutions.* Line 14: The term "strongest dilution" can be misinterpreted. Suggest using "greatest dilution" (lowest concentration) *Agree. In new text these terms are used.*

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28	Institute of Public Health, Slovenia	2. An overview of dermal absorption 2.4. Experimental conditions	The influence of the solvent on skin penetration of a substance may be different for different substances therefore studies should be performed for the preparation, which is not always the case in practice. It is difficult to judge the argumentations of notifiers if we do not have clear rules in which situation a study with active substance would be accepted. Studies performed with three concentrations (product, mix/load dilution and spray dilution) would add a lot to better understanding of dependence of skin penetration on a substance and the solvent present. *Guidance is provided.*
29	ECVAM, DG JRC, European Commission	2. An overview of dermal absorption 2.4. Experimental conditions	Paragraph 2.4, page 5, line 4 of paragraph, and further down in document: SANCO Document 222/2000 states: "At low concentrations the absorbed test substance expressed as percent of applied dose per time interval is in general higher than the percentage absorption at high concentrations. As a consequence, there is no standard absorption percentage for a given substance." It is correctly stated that the bioavailability by dermal route is not a single percent figure, since it depends on the concentration applied, but also on the vehicle, on the surface of contact, on the part of the body which is exposed, on the time of contact, etc. This should be more clearly emphasised. Further in the text the expression "absorption percentage" should be avoided because it intuitively implies a constant ratio, and it is often misunderstood as such. More precise and less concise wording is necessary to avoid this ambiguity. A possible way around this difficulty might be to give a few initial definitions, and refer to them further in the document. For instance, the following concepts (or other as necessary) could be defined (to be discussed by experts): - Dermal absorption rate (how much goes through and into the skin per unit of time and surface), - Amount absorbed (how much has gone through and into the skin after a given time), - Fraction absorbed (amount absorbed, relative to the amount of substance applied on the skin surface), - Fraction bioavailable systemically: (amount of substance which passes into the general circulation, relative to the amount of substance applied on the skin surface). *Issues on kinetics are addressed, bearing in mind that for pesticides % absorption under finite or semifinite conditions, mimicking operator/worker exposure, occurs.*

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30	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	2. An overview of dermal absorption 2.4. Experimental conditions	Please consider the following reference: Buist, H.E., Schaafsma, G. van de Sandt, H., 2009. Relative absorption and dermal loading of chemical substances: Consequences for risk assessment. Manuscript submitted to Regulatory Toxicology and Pharmacology. This document confirms the inverse relation between area dose and percentage of absorption, based on an up-to-date and extensive database. *Done.*
31	French Food Safety Agency, AFSSA	2. An overview of dermal absorption 2.4. Experimental conditions	At the end of § 2.4: *In vitro* dermal absorption studies performed on the active substance (without commercial vehicle/formulants) should not be used to predict dermal absorption from a formulated product, unless the vehicle used in the dermal absorption study is comparable to the preparation' s vehicle *Guidance on this issue is provided.*
32	Charles River Laboratories, UK	2. An overview of dermal absorption 2.4. Experimental conditions	Paragraph 2 line 5: Add: Therefore results should be expressed as both % applied dose and µg/ cm2. *Text has been rewritten.* Paragraph 2, line 12: strongest dilution (I assume this means most concentrated) + last paragraph: "diluted to minimum use concentration". I think this is misleading. Since absorption follows Ficks 2nd Law of diffusion, we should test at least the most concentrated in-use or a range. The most diluted concentration would not cover higher concentrations in line with this Law. *The data show that the greatest dilution generally gives the highest relative absorption.*
33	Technology Sciences (Europe) Limited (TSGE), UK	2. An overview of dermal absorption 2.4. Experimental conditions	Some reference is required either in this section or elsewhere to the metabolic capacity of the test system *in vitro*. For compounds where dermal absorption is influenced by metabolism, adequate metabolic capacity in the test system is required, otherwise absorption may be significantly underestimated. *Issue is discussed in the OP.*
34	Institute of Public Health,	3. Studies on dermal absorption	For the exposure time one value only should be given, preferably 8 hours as the worst scenario of the values suggested now. This is another step to reduce variability in the test procedure. The same holds for point 3.2.

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	Slovenia	3.1. *In vitro* studies	*Agree.*
35	Ministry of Health and Consumer Affairs, Spain	3. Studies on dermal absorption 3.1. *In vitro* studies	Update the first paragraph as follows: The test should be carried out in accordance with "OECD Guideline for the Testing of Chemicals. Guideline 428: Skin Absorption: *in vitro* method" (OECD, 2004) and the OECD Guidance Document for the Conduct of Skin Absorption Studies (OECD, 2004). *Guidance document refers to OECD guidelines.*
36	Federal Office of Consumer Protection and Food Safety, Germany	3. Studies on dermal absorption 3.1. *In vitro* studies	In most studies of this type that we receive, exposure time was 24 hours but not 6 – 8 al-though this would better reflect a normal working day in Europe. Some guidance should be given because the revised guidance document will have an impact on the study design. The question of tape stripping that is sometimes applied also *in vitro*, should be addressed. There is an ongoing discussion whether the flux or the percentage of absorbed radioactivity (including the skin bound portion) should preferably be used. A clearer guidance would be desirable. At least in the OECD expert group, a majority of participants was in favour of the absorbed radioactivity and this approach is also supported by the BfR *Guidance on these issues is provided.*
37	Charles River Laboratories, UK	3. Studies on dermal absorption 3.1. *In vitro* studies	One other minor point is OECD references need updated to 2004 in section 3.1 *Done.*
38	Charles River Laboratories, UK	3. Studies on dermal absorption 3.1. *In vitro* studies	Paragraph 1: OECD, 2004 *Done.*
39	Hellenic Ministry of Rural Development and Food, Greece	3. Studies on dermal absorption 3.1. *In vitro* studies	The revised version (rev. 7) of Guidance Document on Dermal Absorption is very informative and helpful in the derivation of the dermal absorption values in relation to the risk assessment of plant protection products. According to our opinion, the interpretation of data concerning tape stripping in the *in vitro* studies with human skin should be further clarified As it is mentioned in the "EFSA Handbook for the experts' meetings, Section 2: Mammalian toxicology", available in CIRCA, the SCP opinion (Doc. SANCO/222/2000 – rev4 dated 11 April 2001) has stated not

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			to include the amount of substance bound in the stratum corneum to the amount systemically absorbed. This issue has been widely discussed during the PRAPeR meeting leading to the following approach: In the *in vitro* dermal absorption test, the amounts detected on the first two tape strips can be considered as not absorbed (because the substance apparently remains in the stratum corneum) while the amounts on the other tape strips are considered as absorbed. EL agrees to this approach *Guidance on these issues is provided.*
40	Ministry of Health and Consumer Affairs, Spain	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.1 Animal studies	Update the first paragraph as follows (adding the highlighted text): The test should be carried out in accordance with "OECD Guideline for the Testing of Chemicals. Guideline 427: Skin Absorption: *in vivo* method" (OECD, 2004) and the OECD Guidance Document for the Conduct of Skin Absorption Studies (OECD, 2004) *GD refers to OECD guidelines.*
41	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.1 Animal studies	line 8: replace faith with fate. *Document has been re-written.*
42	Charles River Laboratories, UK	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.1 Animal studies	Paragraph 1: OECD, 2004 *References have been updated.*

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43	Dow Agrosciences, UK on behalf of the European Crop Protection Association (ECPA)*, Belgium	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.1 Animal studies	Comment 1: The following text "In order to get insight in the fate of the amount located in the skin, the sampling time should be long enough to determine that absorption from the application site is no longer significant, e.g. until serial non-detects in excreta." should be clarified. Although the original guidance was adequate, it was incorrectly interpreted by some Member States such that the absolute requirement for serial non-detects was the sole criterion for determining whether absorption had ceased. This was never the intention of the original guidance as it was clearly stated that absorption from the application site should in fact "no longer be significant". This is a reasonable requirement. Therefore, the principle should not be lost but the wording needs to be clarified such that the new guidance is totally unambiguous. All that is required is a simple definition of "no longer be significant". ECPA propose the following text: "In order to get insight in the fate of the amount of active substance located in the skin at the application site, the duration of the study should be long enough to determine that absorption is no longer significant. This can be achieved by determination of the amount of active substance excreted per day until the amount eliminated becomes insignificant in relation to the total amount excreted over the duration of the study. In this case, all of the material remaining at the application site should be excluded from the absorbed dose. This approach can be underpinned by consideration of the absorption profile with time, to confirm that absorption had effectively reached a plateau level at the end of the study. If it is not possible to establish that absorption is insignificant, the relationship between the absorbed dose and the residue at the application site during the study should be used to estimate the fraction of the material remaining at the application site that should be used to determine the total absorbed dose." *Guidance on these issues is provided.* *Comment 2:* A specific case worthy of mention in the guidance document relates to bound skin residue (BSR) in the stratum corneum at the application site. "Skin contact with crop protection chemicals can occur during mixing and/or loading and spraying operations. However, systemic uptake of chemicals in contact with the skin is attenuated by the stratum corneum, the principal barrier to dermal penetration and absorption. The stratum corneum is the non-viable outer layer of the skin and is composed of anucleated cells (corneocytes) floating in a lipid matrix. In absence of damage, penetration of chemicals through the stratum corneum is passive and generally via the

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			intercellular lipid lamellae. In general, corneocytes of the stratum corneum are constantly shed and renewed in a process known as desquamation, which lasts approximately 14-21 days (Milstone, 2004; Roberts and Marks, 1980). On occasion, chemicals that contact the skin can become bound to the stratum corneum, the bound skin residue (BSR), which may be lost during the normal process of desquamation or taken up by the capillaries in the viable epidermis and distributed systemically. Generally, if the rate of desquamation is faster than the rate of passive diffusion, the systemic availability of the BSR will likely be negligible. One option to unequivocally resolve the disposition of the BSR, animals, typically rats, should be held post-exposure for 21 days or longer, if required. Evidence of a decline in the total amount of radioactivity eliminated in excreta (urine and/or faces) and expired air (if applicable) must be demonstrated. Milstone, L.M. (2004). Epidermal desquamation. Journal of Dermatological Science 36, 131-140. Roberts, D., and Marks, R. (1980). The determination of regional and age variations in the rate of desquamation: a comparison of four techniques. Journal of Investigative Dermatology 74, 13-16. *The issue is specifically addressed in the GD.*
44	Ministry of Health and Consumer Affairs, Spain	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.2. Human volunteer studies	We consider that the last paragraph should be completed as follows (adding the following text): "Results from field studies, if well conducted, and especially biomonitoring data may be helpful to confirm results obtained from experimental dermal *in vivo* and *in vitro* testing, and should be considered when it comes to estimating the dermal penetration of a substance. Additionally, even if there is not a field study carried out with the substance of interest, but there is one conducted with a chemically related substance that has similar physicochemical properties, the obtained results should be taken into account". For example, a recent study has evaluated exposure and occupational risk during manual operations with ornamental plants treated with omethoate in intensive cultivation tunnels (Aprea, 2005). The plants had been treated 37 h before reentry with 220 ml of a commercial product (565 g/l pure omethoate) dispersed in 200 l water (equivalent to 0.62 g/l of active ingredient).The urinary excretion of alkylphosphate allowed for estimating that the fraction of omethoate absorbed through the skin during work was about 16.5%. Omethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorothioate) is a metabolite (the oxygen analogue) of dimethoate: O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate; both compounds have very similar physico-chemical properties (omethoate: mw 213 and logPow -0.7; dimethoate: mw 229 and logPow 0.7). In fact, the octanol–water partition coefficient of omethoate would suggest a more unfavourable absorption compared to dimethoate. However, in the conclusion regarding the peer review of the pesticide risk assessment of the active substance dimethoate (EFSA, 2006) the dermal absorption established is 0.15% for the concentrate and 2.0% for the dilution.

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			These values come from the combination of rat *in vitro* and *in vivo* data and human *in vitro* data (Davies, D. J., 1999; Heylings, 2000; Leibold, E., 2001), but interestingly, in the dermal penetration *in vitro* study using human membranes, at the end of an 8 hour exposure with a spray-strength dilution of 2 g dimethoate/l, the absorption of dimethoate was 14,5%. This outcome suggests that the conclusion laid down in EFSA's document could represent an underestimation. Literature references: OECD Environmental health and safety publications. Series on testing and assessment n 28. Guidance Document for the Conduct of Skin Absorption Studies; March 2004. OECD Guideline for the testing of chemicals: 428. Skin absorption: *in vitro* method. Adopted : 13 April 2004. OECD Guideline for the testing of chemicals: 427. Skin absorption: *in vitro* method. Adopted : 13 April 2004 C. Aprea, L. Centi, S. Santini, L. Lunghini, B. Banchi, G. Sciarra. Exposure to Omethoate During Stapling of Ornamental Plants in Intensive Cultivation Tunnels: Influence of Environmental Conditions on Absorption of the Pesticide. Arch. Environ. Contam. Toxicol. 49, 577–588 (2005). EFSA Scientific Report (2006) 84, 1-102, Conclusion on the peer review of dimethoate. Davies, D.J., 1999. Dimethoate: *in vitro* absorption from a 400 g/l EC formulation through human and rat epidermis. Company Report No. JV1591/REG/REPT. DTF Doc No. 460-003. Unpublished. Heylings, J.R., 2000. Dimethoate: *In vitro* absorption from a 400 g/l EC formulation through human and rat epidermis. Statement regarding project no.: 104-065 contract CO9027 - JV1591. Company Report No. CO9027-JV1591. DTF Doc No. 481-036. Unpublished. Leibold, E., Hoffmann, H.D., 2001. Study on the dermal penetration of 14C-Dimethoate in rats. Company Report No. 01B0418/006016. DTF Doc No. 654-002. Unpublished. *Consideration of field studies is mentioned in the GD and in OP.*
45	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.2. Human	This text needs to be updated. *Done.*

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	behalf of the Netherlands	volunteer studies	
46	Technology Sciences (Europe) Limited (TSGE), UK	3. Studies on dermal absorption 3.2. *In vivo* studies 3.2.2. Human volunteer studies	The section needs to be modified and clarified, in line with the position on the use of human volunteer studies. *See comment 4.*
47	ECVAM, DG JRC, European Commission	4. Decision making process for setting dermal absorption percentages General comments	General comment on Chapter 4: fate of compound/stratum corneum, skin reservoir: There is a need for a standard (tiered?) procedure to interpret the possible role of reservoir of the stratum corneum. A recurrent discussion point in regulatory meetings is the number of strips which should be considered as non-absorbed when doing a tape strip procedure at the end of a dermal absorption study. There is also some disagreement in the available guidance or literature: For *in vitro* tests, the SCCP statement in their 2006 opinion (SCCP/0970/06) is that "the amount present in the stratum corneum at the time of sampling is considered as not contributing to the systemic dose." Yourick et al. (2004) on the same topic seem to have an opposite view (p. 318): "The above examples demonstrate that the amount of material remaining in the skin at the end of a study should be included as part of the total dose absorbed (as recommended by Bronaugh and Collier, 1991), unless the fate of the chemical in skin is investigated and it is shown not to be available for systemic absorption." Possibly the approach could be linked to the lipophilicity of the substance in question? It is usually considered that the stratum corneum is the most effective barrier against hydrophilic compounds, and the living layers of the epidermis and dermis against lipophilic compounds. References: 1. Jeffrey J. Yourick, Michael L. Koenig, Debra L. Yourick, and Robert L. Bronaugh. Fate of chemicals in skin after

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			dermal application: does the *in vitro* skin reservoir affect the estimate of systemic absorption? Toxicology and Applied Pharmacology 195 (2004) 309–320. 2. SCCP/0970/06. Scientific Committee on Consumer Products Opinion on basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients - updated March 2006. Adopted by the SCCP during the 7th plenary of 28 March 2006. *Guidance is provided.*
48	Swedish Chemicals Agency (KemI)	4. Decision making process for setting dermal absorption percentages General comments	Dermal absorption values should be given as integers and numbers below 1% should be rounded to 1%. This would compensate for scratches and small wounds on the farmers skin, which would represent a larger percentage of a low figure than a high figure. Furthermore, the accuracy of the studies does not allow setting dermal absorption figures with decimal precision. *The issue of rounding has been addressed. See also comment 2.*
49	Swedish Chemicals Agency (KemI)	4. Decision making process for setting dermal absorption percentages General comments	Clear instructions on how to deal with substances found in stratum corneum are needed. When should tape strips be used (*in vivo*/*in vitro*), how many strips should be excluded etc. In this respect, as the number of tapes stripped seem to vary between studies, it could be a point to add that regardless of the number of tapes stripped, the outer numbers excluded should be the same, if equal conditions are assumed, and so it is better to suggest a number of tape strips to exclude instead of a number to include. *Guidance is provided.*
50	Technology Sciences (Europe) Limited (TSGE), UK	4. Decision making process for setting dermal absorption percentages General comments	Figure I (page 10) consistency is required in the use of "rat" or "animal" Two boxes "*in vivo* studies" are not required. The two options under the second "*in vivo* studies" box should be consistent: (1) no serial non-detects and no strong decline (2) serial non-detects or strong decline Box at bottom right [dermal absorption percentages following *in vitro* studies] indicates that the value should be calculated using the skin plus receptor medium. There is no option here for refining the value by discounting all or

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			some of the skin residue using expert judgement, as stated earlier in the document *Text and figures have been re-drafted.*
51	Federal Office of Consumer Protection and Food Safety, Germany	4. Decision making process for setting dermal absorption percentages 4.1. Dermal absorption based on default values	It is further supported that dermal absorption must not be derived from a comparison between oral and dermal acute studies as outlined in the current document. However, the situation may be different when subacute studies are considered. This principal opportunity should be mentioned and conditions should be described under which a comparison might contribute at least to a rough estimate. *Guidance is provided.* The opinion that dermal absorption, usually, will not exceed the oral one may be right but it should be clarified what the scientific basis for this assumption actually was. In the OECD expert group, this argument was not accepted and dermal absorption should not be esti-mated on the basis of oral data. *Guidance is provided.* Rather than only providing a reference to literature, the experimental evidence for choosing either 10 or 100 % default values based on molecular mass and log POW should be better represented and more extensively discussed along with the uncertainties associated with this approach. *New default values with justification are provided.*
52	Pesticides Safety Directorate, United Kingdom	4. Decision making process for setting dermal absorption percentages 4.1. Dermal absorption based on default values	Page 7 - lines 8 -15: The scientific basis for the log Pow and molecular weight criteria is questionable. There is also a large margin from 100% to 10% it is possible that some intermediate value e.g. 30% might be justifiable based on alternative criteria. The choice of these default values should be reviewed to see if they are justified scientifically. A basis for any investigation could be the studies submitted during the 91/414 review process. *New default values with justification are provided.*

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53	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	4. Decision making process for setting dermal absorption percentages 4.1. Dermal absorption based on default values	first paragraph: It should be realized that only few compounds (evaluated within the framework of 91/414/EC) exist with the physico-chemical properties leading to the default of 10% absorption. Since 1999, considerable progress has been made in the area of QSARs and it would be useful to provide some up to date information (see e.g. T. Bouwman, M.T.D. Cronin, J.G.M. Bessems, J.J.M. van de Sandt (2007). Improving the applicability of (Q)SARs for percutaneous penetration in regulatory risk assessment. Human and Experimental Toxicology 27. 269-276, 2007). *Issue is considered in the GD.* second paragraph (oral absorption/ADME study): The assumption that dermal absorption is not likely to exceed oral absorption is indeed applied in current risk assessments. However, this paragraph refers only to oral absorption determined in bile cannulation studies. Oral absorption can also be determined based on an absorption study in non-cannulated animals. In case it is clear that a substance is almost exclusively excreted via urine and excretion via bile is relatively small, and the oral absorption has been determined in non-cannulated animals, the assumption that dermal absorption is not likely to exceed oral absorption can still be made. Please add this to the Guidance Document. *Issue is addressed in both the Opinion and the GD.*
54	Charles River Laboratories, UK	4. Decision making process for setting dermal absorption percentages 4.1. Dermal absorption based on default values	Paragraph 1, line 6-10: In practice, I have rarely seen values greater than 50% for absorption, perhaps a slightly less conservative value could be suggested eg 75% -80%. *New default values with justification are provided.*
55	Federal Office of Consumer Protection and Food Safety,	4. Decision making process for setting dermal absorption percentages	There are not so few examples of the EU or individual MS having established the dermal absorption rate only on the basis of results obtained with human skin samples. It should be emphasised that this is considered usual practice in the EU and scientific evidence to sup-port this approach should be given, as well as possible limitations

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	Germany	4.2. Dermal absorption based on *in vitro* human and rat studies	*Human in vitro proposed as stand alone dataset*.
56	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	4. Decision making process for setting dermal absorption percentages 4.2. Dermal absorption based on *in vitro* human and rat studies	In dermal absorption studies (*in vitro* and *in vivo*) tape stripping can be performed. It would be useful to add a paragraph to provide some more tools for the risk assessors how to deal with tape stripping data. What number of tape strips can be regarded as non-absorbed in *in vivo* and *in vitro* studies? It has to be noted that the time of tape stripping is important. For instance, tape stripping immediately following washing of the skin may remove test substance that might have been absorbed through the skin, thus possibly leading to an underestimation of the dermal absorption. Test substance removed from the skin by tape stripping 24h after washing is less likely to have been absorbed. However, also a general warning should be given regarding the use of tape stripping data, given its limitations such as variability and the absence of general guidance. Since this issue will also dealt within the OECD guidance document (mentioned above; comments on the first concept have been received and the document is currently under revision) a reference to this document may suffice. In the current EU risk assessments of Plant Protection Products, it has been decided in the PRAPeR meetings to use the pragmatic approach that the first two tape strips in an *in vitro* study can be regarded as not absorbed (See the EFSA List of decisions for mammalian toxicology). (The problem however is that in many study reports the results of the individual tape strips are not reported) *Proposal for guidance for tape stripping is provided in the GD.*
57	Swedish Chemicals Agency (KemI)	4. Decision making process for setting dermal absorption percentages 4.2. Dermal absorption based on *in vitro* human and	The word 'skin' is frequently used in the document. A recurring problem is whether stratum corneum, or parts of it, should be included or not. It should therefore be clearly stated which layers of the skin are referred to when the word 'skin' is used. (Example, top paragraph, page 8, line 5: 'By including the amount retained in the skin *in vitro*, a more acceptable estimation of skin absorption can be obtained.') *A glossary is provided.*

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		rat studies	
58	French Food Safety Agency, AFSSA	4. Decision making process for setting dermal absorption percentages 4.2. Dermal absorption based on *in vitro* human and rat studies	1st paragraph: "There is an increase in the number…If refinement is needed, it should be convincingly demonstrated that the skin dose does not become absorbed at a later stage." Comment to add: Continuous desquamation makes the first layers of the stratum corneum unlike to be part of this reservoir. Therefore, it is generally admitted that the 2-3 first strips are not included in the absorbable dose. The deeper layers and the remaining skin are included, unless it is demonstrated that they should not. *Proposal for guidance for tape stripping is provided in the GD.* 2nd paragraph: "The maximum flux at relevant……within one species (provided they are tested under otherwise identical and relevant test conditions)". This paragraph could perhaps be more detailed, to allow a pertinent use of the maximum flux value. *Guidance for the use of flux or % absorbed is provided.*
59	Charles River Laboratories, UK	4. Decision making process for setting dermal absorption percentages 4.2. Dermal absorption based on *in vitro* human and rat studies	Paragraph 1, line 11: The major issue identified here is as a result of the way that data is generated in the two test systems (*in vitro* versus *in vivo*). Historically, skin was removed from the rat and analysed. There was no consideration taken as to the stratum corneum/ epidermis/ dermis distribution. The *in vitro* studies always have at least separated the "dead" stratum corneum from the "living" skin and often have separated the layers of stratum corneum and the epidermis/ dermis. Indeed, we always take individual tape strips (1-20) to provide a stratum corneum profile. This adds information on whether the penetrant is on or near the surface of the stratum corneum and hence will be sloughed off or if it is uniformly distributed throughout the stratum corneum so the stratum corneum should then be added to the skin values as this material is clearly labile. The latter example then brings the *in vivo*/ *in vitro* studies closer together. We also perform our *in vivo* studies now in line with the *in vitro* studies, i.e. we remove the separate layers of the stratum corneum by tape stripping rather than taking the skin as a single sample. A closer correlation is then possible.

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			Guidance on tape stripping is provided. Last line: the stratum corneum profiling will allow demonstration that the "skin" dose does not become absorbed. The material in the "living" skin tissue (viable epidermis and dermis" should always be included in the risk assessment cf para 2, line 1, section 2.1, dermal absorption definition (EPA, 1992). *Agree. This is discussed in the text and guidance is provided.* Paragraph 2: The maximum flux comparison is of course useful, but there are other important parameters to compare eg total absorption and skin distribution (as µg/cm2), and the shape of the absorption/ stratum corneum profiles. This is especially important in comparing species where the kinetics may be different. *Agree. This is discussed in the text and guidance is provided.*
60	Technology Sciences (Europe) Limited (TSGE), UK	4. Decision making process for setting dermal absorption percentages 4.2. Dermal absorption based on *in vitro* human and rat studies	Page 8, 2nd paragraph, line 2. It should be clarified that the maximum flux can ALSO be used for comparison of dermal absorption between species - a comparison can also be given using amounts in the receptor fluid (with or without skin) as described in the previous paragraph. *Agree. This is discussed in the text and guidance is provided.*
61	Federal Office of Consumer Protection and Food Safety, Germany	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	In this section, the most difficult question is on the termination of absorption that continues over the post-observation time. The "serial non-detects approach" should be re-examined because it failed to find world-wide acceptance so far. Furthermore, the most relevant time points should be defined that should be used for deriving dermal absorption values to be used in risk assessment (e.g., 8 – 10 hr measurements vs. determination after 24 hrs). Or, as an alternative, should the numerically highest value be used in any case? *These issues are discussed in the text and guidance is provided.*
62	Pesticides Safety Directorate,	4. Decision making process for setting	Page 8 - line 6 - 12 of section 4.3.

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	United Kingdom	dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	The text regarding completion of excretion and serial non-detects should be deleted as it can be an invitation to do poor studies. At present this text is being interpreted very strictly by some MS (ignoring the subsequent comment about clear decrease in excretion) such that a study with excretion levels at about the LoQ (limit of quantification) at the end of the study has not shown complete excretion and thus the skin depot is deemed as being included. One way round this is to do the study with radio-label of a low specific activity and hence a high LoQ. A preferred approach would be to stipulate that the duration of an *in vivo* study should be such that it is the longer of 96 hours or four times (??) the terminal excretory half life from the oral ADME study. If at the end of such a dermal study there is a clear demonstration that excretion has shown a clear decline (or some arbitrary value e.g. 90% of the absorbed dose has been excreted) then the residue at the application site can be excluded from the bioavailable fraction. *These issues are discussed in the text and guidance is provided.*
63	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	See identical comment under Section 4.2. (Comment 53) In dermal absorption studies (*in vitro* and *in vivo*) tape stripping can be performed. It would be useful to add a paragraph to provide some more tools for the risk assessors how to deal with tape stripping data. What number of tape strips can be regarded as non-absorbed in *in vivo* and *in vitro* studies? It has to be noted that the time of tape stripping is important. For instance, tape stripping immediately following washing of the skin may remove test substance that might have been absorbed through the skin, thus possibly leading to an underestimation of the dermal absorption. Test substance removed from the skin by tape stripping 24h after washing is less likely to have been absorbed. However, also a general warning should be given regarding the use of tape stripping data, given its limitations such as variability and the absence of general guidance. Since this issue will also dealt within the OECD guidance document (mentioned above; comments on the first concept have been received and the document is currently under revision) a reference to this document may suffice. In the current EU risk assessments of Plant Protection Products, it has been decided in the PRAPeR meetings to use the pragmatic approach that the first two tape strips in an *in vitro* study can be regarded as not absorbed (See the EFSA List of decisions for mammalian toxicology). (The problem however is that in many study reports the results of the individual tape strips are not reported) *Tape stripping is specifically addressed in the GD and guidance is provided.*

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64	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	first sentence: OECD 428 should be OECD 427 In an *in vivo* study, measurements are performed at different time points (e.g. after 24 h, 48 h, 168 h). Currently, different approaches are taken by Member States to evaluate these *in vivo* results: some Member States calculated dermal absorption based on the results after 24 h, while others calculate the worst-case values (e.g. based on results after 168 h). Is it possible to provide guidance on the time point which should be used to determine dermal absorption: the values after 24 h, 48 h, 72 h, 168 h, or the worst-case values (taking into account that the AOEL is expressed as acceptable exposure per 24 hours)? Guidance on normalization should be included (for both *in vitro* and *in vivo* studies) in case the recovery is <90% (with an acceptable justification for the relatively low recovery). Should the results be corrected for the lower recovery? And if yes, how (e.g. 'normalise' the results to 100%: 8% absorption with a recovery of 85% would be 9.4%)? This can probably only be resolved on a case-by-case base, depending on the cause of the low recovery, but some examples could be illustrative. This issue will also be dealt with within the OECD guidance document (mentioned above). *Guidance on this issues is provided in the GD.*
65	French Food Safety Agency, AFSSA	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	This comment can also be found in section 4.2 (comment 55) Continuous desquamation makes the first layers of the stratum corneum unlike to be part of this reservoir. Therefore, it is generally admitted that the 2 first strips are not included in the absorbable dose. The deeper layers and the remaining skin are included, unless it is demonstrated that they should not. *Extensive guidance on tape stripping is given.*
66	Charles River Laboratories, UK	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	First paragraph line 1: Replace OECD 428 (OECD 200b) with OECD 427 (2004b): *Text has been rewritten.* First paragraph line 8 and last sentence:: Having information on the stratum corneum profile will allow a simpler interpretation of the data leading to a rational

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			data based argument for including/ rejecting the stratum corneum in the risk assessment. The living epidermis and dermis must always be included. *Criteria relating to the exclusion of the stratum corneum are provided .*
67	Dow Agrosciences, UK on behalf of the European Crop Protection Association (ECPA), Belgium	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	Comment 1: Replace "If sampling is done over a sufficiently long period of time (e.g. until serial non-detects in excreta) the amount detected in the application site after washing should not be included in the amount absorbed." with text based on the rationale for determining the end of absorption contained in ECPA Comment 1 in Section 3.2.1: "In order to get insight in the fate of the amount of active substance located in the skin at the application site, the duration of the study should be long enough to determine that absorption is no longer significant. This can be achieved by determination of the amount of active substance excreted per day until the amount eliminated becomes insignificant in relation to the total amount excreted over the duration of the study. In this case, all of the material remaining at the application site should be excluded from the absorbed dose. This approach can be underpinned by consideration of the absorption profile with time, to confirm that absorption had effectively reached a plateau level at the end of the study. If it is not possible to establish that absorption is insignificant, the relationship between the absorbed dose and the residue at the application site during the study should be used to estimate the fraction of the material remaining at the application site that should be used to determine the total absorbed dose." *These issues are addressed in the GD and guidance is provided.* *Comment 2:* Delete the paragraph: "In case excretion of the substance and/or its metabolites has not come to an end within the sampling period, but there are indications of a clear decrease in excretion, only a part of the skin bound dose may be included in the absorption by expert judgement (Thongsinthusak, 1999 ; De Heer, 1999). In case the experiment is terminated before serial non-detects in excreta are observed and/or no clear decline in excreta is measured, the amount located in the skin should be considered as being absorbed (Chu, 1996) (see Figure 1)" based on the rationale for determining the end of absorption contained in ECPA Comment #1 in Section 3.2.1, as shown above. *These issues are addressed in the GD and guidance is provided.*

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			Comment 3: New text: In the absence of sufficient experimental data to adequately define the end of absorption the following approach may be considered. "Dermal absorption is defined as the amount of applied dose that is ultimately taken up systemically and eliminated via urine, faeces and/or expired air, and that includes the amount recovered in tissue and carcass at termination. However, systemic uptake of chemicals in contact with the skin is attenuated by the stratum corneum, the principal barrier to dermal penetration and absorption. On occasion, chemicals can become bound to the stratum corneum, the bound skin residue (BSR), and unless its disposition is resolved, it must be assumed to be absorbed. The BSR may be lost during the normal process of desquamation or taken up by the capillaries in the viable epidermis and distributed systemically. Generally, if the rate of desquamation is faster than the rate of passive diffusion, the systemic availability of the BSR will likely be negligible. Therefore, sampling of excreta (urine, faeces, expired air) must be done over a sufficiently long period of time to resolve disposition of the BSR (i.e., up to 21 days post exposure, which encompasses the period of desquamation); a plot of cumulative radioactivity eliminated (via excreta) in maximized aliquots of excreta from analysis by liquid scintillation counting will provide the best practice for demonstration of a decline in absorption (from the BSR). However, for rat *in vivo* dermal studies terminated before a sufficient period of time post-exposure needed to resolve disposition of the BSR, a modeling technique can be employed to estimate the maximum absorption, and therefore, the systemic availability of the BSR (Thongsinthusak et al., 1999)." The Thongsinthusak et al. (1999) exponential saturation model was validated against a published dermal absorption study in human volunteers of 12 pesticides (Feldmann and Maibach, 1974). Overall, the model-derived dermal absorption estimates determined by the model were consistent with the reported values in Feldmann and Maibach (1974) and provided more realistic (yet conservative) estimates for those pesticides where the terminal elimination half-life ($t_{1/2}$) for urinary excretion was greater than the $t_{1/2}$ for dermal absorption. Accuracy of the maximum absorption prediction by the model is dependent on the elimination rate (via excreta) being faster than the absorption rate from the BSR to the systemic circulation. When the only source of available dose is that contained in the stratum corneum as the input (the BSR), and the elimination rate or output (via excreta) of the systemic dose is faster than the absorption rate or the input from the BSR, and there's no evidence of binding of the chemical or its metabolites to tissues once the chemical enters the body, then BSR depletion, either into the body or its loss via desquamation, will be directly reflected in the excreta output. However, if the chemical binds to tissues, as either the parent chemical or a metabolite, which in turn slows the overall rate of elimination from the body (output<input), the model losses the ability to resolve elimination from the BSR alone. In this case, the model will predict maximum absorption to occur long after the BSR has been lost by the normal process of desquamation (14-21 days) and the extent of absorption at the asymptote is suspect. Reason and justification for new text, if necessary: Removed concept of "serial non-detects" and provide additional guidance and clarification on resolution of BSR. References:

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			Feldmann, R. J., and Maibach, H. I. (1974). Percutaneous penetration of some pesticides and herbicides in man. Toxicology and Applied Pharmacololgy 28, 126-132. Thongsinthusak T., Ross J.H., Saiz S.G., and Krieger R.I. (1999). Estimation of Dermal Absorption Using the Exponential Saturation Model. Regulatory Toxicology and Pharmacology 29, 37-43. *This issue is addressed in the GD and guidance is provided.*
68	Institute of Public Health, Slovenia	4. Decision making process for setting dermal absorption percentages 4.3. Dermal absorption based on *in vivo* data	There is great variability in expert judgments on the active substance in stratum corneum layers to be included or excluded as the amount absorbed. More guidance would be recommended in this document *Guidance is provided.*
69	Institute of Public Health, Slovenia	4. Decision making process for setting dermal absorption percentages 4.4. Dermal absorption percentage based on *in vivo* rat studies in combination with *in vitro* data	If the usefulness of the Kp for dermal risk assessment is limited, this should not be included in predictions of absorbed dose. The equation would be preferred also for additional options of use (different formulations). *Kp is not considered for prediction.*
70	Federal Office of Consumer Protection and Food Safety, Germany	4. Decision making process for setting dermal absorption percentages 4.4. Dermal absorption percentage based on	A clear recommendation to use this so-called triple pack, if valid studies are available, should be given. *Based on the available date use of in vitro human data as stand alone is proposed as a first option. The" triple pack" approach is addressed in the GD.*

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		in vivo rat studies in combination with *in vitro* data	
71	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	4. Decision making process for setting dermal absorption percentages 4.4. Dermal absorption percentage based on *in vivo* rat studies in combination with *in vitro* data	This paragraph describes that *in vivo* dermal absorption data in the rat may be adjusted in the light of the relative absorption through rat and human skin *in vitro*. It should be clearly stated here that the conditions under which the *in vitro* studies with human and rat skin were performed should be similar, for instance with respect to concentration of the test substance, vehicle used, receptor fluid, duration of test/ stripping etc. Page 9, last sentence: This should be rephrased: 'Similar adjustments can be made for differences between the dermal absorption of an active substance and the dermal absorption of an active substance in combination with (a) formulant(s) (e.g. *in vivo* active substance in rat and *in vitro* rat data on a formulant+active substance and active substance). *All these issues are addressed in the GD and guidance is provided.*
72	Charles River Laboratories, UK	4. Decision making process for setting dermal absorption percentages 4.4. Dermal absorption percentage based on *in vivo* rat studies in combination with *in vitro* data	This method is certainly a useful approach to predict absorption in the human *in vivo* situation. However, this approach needs to be carefully considered. For example, the study design, formulation, test item concentration etc must be identical in the 3 tests used to generate *in vivo* absorption. Also, a realistic absorption value is needed and not an over prediction. Ethanol: water receptor fluids frequently generate an over prediction of absorption and provided an acceptable MoS is obtained, this does not matter. However, the overprediction is accentuated in the rat *in vitro* compared to the human *in vitro* and as such will result in a smaller calculated *in vivo* human value. *All these issues are addressed in the GD. The basis for the comment regarding ethanol water receptor fluids is unclear.* Figure 1.: Dermal absorption percentages: must always include viable skin, but not stratum corneum (ie the barrier), some or all of the stratum corneum may be added where absorption is in steady state (absorption profile) or if the stratum corneum profile indicates test item is unlikely to be sloughed off. *These issues are addressed in the GD and guidance is provided.*
73	Technology Sciences (Europe) Limited (TSGE), UK	4. Decision making process for setting dermal absorption percentages	Page 9 line 9 It is stated that the "dermal absorption percentage" (receptor medium plus skin dose)" can be used to determine a dermal value. However it is stated in section 4.2 that the skin dose need not be included if it can be demonstrated that this does not become absorbed at a later stage.

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		4.4. Dermal absorption percentage based on *in vivo* rat studies in combination with *in vitro* data	*This issue is addressed in the GD and guidance is provided.* The term "skin dose" is misleading, suggest using "skin depot" or "skin residue" for clarification. *A glossary has been provided.*
74	Pesticides Safety Directorate, United Kingdom	5. Proposal for a tiered approach to risk assessment for operator exposure, using default dermal absorption percentage or dermal absorption percentage determined experimentally	Figure 2: In order to reduce the use of animals without compromising the overall evaluation, the first stage in Tier 3 should be to take the values from well performed studies using human skin *in vitro*. If these give an acceptable outcome then there is no need for any further studies. Rat studies *in vitro* over estimate human values so there is no need to do rat *in vitro* studies unless there is a need to do a rat to human comparison using *in vivo* & *in vitro* data. *See comment 70.*
75	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	5. Proposal for a tiered approach to risk assessment for operator exposure, using default dermal absorption percentage or dermal absorption percentage determined experimentally	Since this document does not cover the risk assessment process, it is advised to remove chapter 5 and Figure 2. Furthermore, Figure 2 is not complete with regard to the risk assessment process and does not add relevant information to the guidance on dermal absorption. *Done.*
76	Technology Sciences (Europe) Limited (TSGE), UK	5. Proposal for a tiered approach to risk assessment for operator exposure, using default dermal	Figure 2 page 12 Tier II indicates that a dermal absorption value based on *in vitro* data should be based on "receptor medium plus skin dose". This excludes the possibility of expert judgement regarding the likely systemic availability of the skin dose, as outlined earlier in the document.

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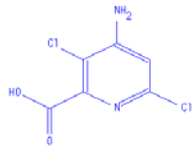
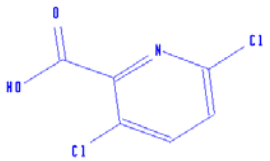
		absorption percentage or dermal absorption percentage determined experimentally	The figure also needs to include an option for refining the default dermal absorption values based on the extenet of oral absorption, as detailed in Section 4.1 *Guidance on these issues is provided.*
77	Board for the Authorisation of Plant Protection Products and Biocides (CTBG), on behalf of the Netherlands	6. Comments for additional chapters	Please add an additional chapter on tape-stripping (see also NL comment on 4.2 and 4.3). *Done.*
78	Pesticides Safety Directorate, United Kingdom	6. Comments for additional chapters	<u>Area where there is a need for additional guidance include:</u> 1.Extrapolating dermal absorption of an active substance from one formulation type with data to another with no data. Some formulation types give lower dermal absorption values than others e.g. granule less than an EC. It is therefore feasible to take the data from the EC and use the value for a granule. However the reverse is not necessarily applicable. PSD has a simple matrix that it uses (see http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/Regulatory_Update_15-2005_AnnexA_Tables_Flow-chart(1).pdf) but note the caveats that apply due to the uncertainties of such an approach. It is possible that such an approach might not be acceptable to all MS in which case some alternate guidance e.g. "no extrapolation is possible" would make it clear to applicants that formulation specific data are required if default values are not to apply. In order to achieve annex 1 listing under 91/414, only one lead product needs to be considered. Providing guidance on how to use data on the dermal absorption of the lead product for other products could increase consistency of assessments for registration across member states. *Guidance on when it is possible to extrapolate between formulations is provided.* 2.Even though it is clearly mentioned in the OECD test guideline, *in vitro* dermal absorption studies are still being performed without adequate confirmation of the active substance solubility in the receptor fluid. SOme text addressing this should be provided along the lines of: - " for active substances with low water solubility (e.g. <100mg/L) solubility in the receptor fluid should be demonstrated to be greater than 10 times the amount diffusing into the receptor fluid

No	Institution	Chapter	Stakeholder comment and PPR Panel response
			during the study.". If the active substance is poorly soluble in the receptor fluid, the results of the study can be significnaly influenced by the limiation on the solubility. *Addressed in the GD and guidance is provided.* 3. The current text has no guidance on tape stripping. Clear guidance should be developed on the performance of tape stripping e.g. strips should be analysed individually so that the profile within the stratum corneum can be determined. Also on which tape strips can be considered to represent non-available material under all circumstances e.g. the first 3 strips and those which might be excluded if there is clear evidence of absorption and excretion declining (see previous comments on section 4). *Done.* 4. It could be useful to provide guidance on how to deal with poor recoveries under different conditions. For example if all animals / wells have a recovery of <92% and the dermal absorption value is 3% is it valid to assume all the missing material could have been absorbed - this could make a 3 fold difference to the result? Instead of adding in all the missing material to the absorbed fraction is it better to multiply all values up ass by 100/92? If one or two samples have low recoveries but others are ca 100% it is possible to compare the high and low recovery samples to see where the losses might be occurring. Under what circumstances can recoveries be considered to be so poor that the results are meaningless and the study should be discarded? *Addressed in the GD and guidance is provided.*
79	French Food Safety Agency, AFSSA	6. Comments for additional chapters	We propose to add a section to Point 4 "4.5 Dermal absorption based on studies conducted on other formulations" When specific experimental data are missing, available information from other products can be used to predict the dermal absorption rate in the evaluated product. We suggest a possible guidance to help the assessor to decide the basic rules where the extrapolation is acceptable. Examples are given below: - differences on the a.s. concentration (same type of formulation and same vehicle)Extrapolations are possible for the diluted (if similar dilutions) and for the concentrate (if similar concentrations). - vehicle is different (same a.s concentration and same type of formulation)For liquids, if the (main) solvents belong to the same chemical family, extrapolation are acceptable for both diluted (same in-use concentrations) and concentrate.Concerning solid formulations with different inerts and formulants of same chemical family, extrapolation are possible for both diluted (same in-use concentrations) and concentrate.

No	Institution	Chapter	Stakeholder comment and PPR Panel response
			different type of formulations (same a.s concentration and similar vehicle/inerts) WP WDG yes, for both concentrate and diluted, SC WP, WDG yes for diluted EC, SC, SL, WP, WDG, (concentrate)GR yes = worse case *Addressed. See comment 78 (1)*
80	Charles River Laboratories, UK	6. Comments for additional chapters	General suggestions: There is very little discussion on the issue of the stratum corneum. There is no discussion on Ficks 2nd Law of diffusion. Since absorption is Ficks 2nd Law of diffusion, it is important to define what is the semi-permeable membrane. It is generally accepted that the membrane is actually the stratum corneum. This makes it essential to include the "viable" skin, but not the stratum corneum in the risk assessment (in line with the citation for EPA, 1992). However, it does not take into account the potential stratum corneum reservoir. With stratum corneum profiling detailed, it is much simpler to decide if all/ some/ none of the stratum corneum should be included in the risk assessment especially if compared with the absorption profile. *Tape stripping and the relevance of the stratum corneum are discussed extensively.*
81	Dow Agrosciences, UK on behalf of the European Crop Protection Association (ECPA), Belgium	6. Comments for additional chapters	Re-entry worker exposure: Under conditions of Good Agricultural Practice, workers re-entering a treated field may be exposed to treated plants and thus to dried residues of the active ingredient present on the plant surface. In dermal absorption studies, normally two or three concentrations are tested, i.e. - the undiluted formulation to mimic exposure during mixing/loading (high skin area concentration), and -1 or 2 aqueous spray-strength dilutions to mimic exposure during spray application of the diluted product (low skin area concentration). Guidance is required on how to derive dermal absorption estimates to adequately reflect the exposure scenario for re-entry workers. This will require guidance on experimental testing to cover re-entry worker exposure. *This issue is addressed in the GD and guidance is provided.*
82	Dow Agrosciences,	6. Comments for additional chapters	Comment - Read Across Guidance on the concept of '*Read Across'* is required as under 91/414 dermal absorption values are normally generated

No	Institution	Chapter	Stakeholder comment and PPR Panel response
	UK on behalf of the European Crop Protection Association (ECPA), Belgium		for an active substance in a single formulation type. Registration of the active substance under Annex III may require modified or different formulation to fulfil specific Member State agricultural requirements to meet the needs of the different application systems, crop or target pest. *Read Across* was developed under the REACH directive for chemical categories or analogues based on the similarities of physico-chemical properties and structural similarities. However, for PPPs, unlike chemicals assessed under REACH, the impact of formulation type on the dermal absorption of the active substance must be taken into consideration. *Read Across* can be applied to: 1) new active ingredient or 2) modified or new formulation type for an existing registered active substance. 1. New Active Ingredient In the absence of experimental data on a new active, a Tier One assessment can be made based on the similarity of an existing registered active(s), to indicate whether an active substance can be assigned default dermal absorption values of 1%, 5%, 10%, 25% or 50%. An example of the applicability of this approach is presented in Table 1 where the dermal absorption of either active can be predicted from the other. **Table 1: Potential for Read Across based on Physico-chemical Properties**

No	Institution	Chapter	Stakeholder comment and PPR Panel response

Structure		
Compound	1	2
Molecular weight	207.16	192
Water Solubility	200 g/L	142 g/L
Log K_{ow}	-2.87	-2.63
Formulation Type	Oil in Water* Emulsion	Soluble Liquid
Concentration	30 g/L	97.5 g/L
Absorption Formulation	1.70% (3% **)	2.36% (3.23%**)
Spray Concentration	0.446 g/L	0.237 g/L
Absorption Spray Dilution	2.91% (4.8%**)	1.48% (3.2%**)

*active in water phase ** including Stratum Corneum

This issue is addressed in the new GD. See comment 78 (1)

2. Modified or New Formulation Type

The impact of a change of formulation type on the dermal absorption of an active substance can be summarised in an approach based on UK PSD Guidance for re-registration of actives and is summarised in Table 2.

Table 2: Impact of Formulation Change on Dermal Absorption Values

No	Institution	Chapter	Stakeholder comment and PPR Panel response

Registered Product	New Formulation: EC	SL	Oil * /water	Water*.oil	SC/WP/WDG	Bait	In Use Granule
EC	Similar	Similar	Similar	Lower	Lower	Lower	Lower
SL	Higher	Similar	Higher	Similar	Lower	Lower	Lower
Oil*/water	Similar	Higher	Similar	Higher	Lower	Lower	Lower
Water*/oil	Higher	Similar	Higher	Similar	lower	Lower	Lower
SC/WP/WG	Higher	Higher	Higher	Higher	Similar	lower	Lower
Bait	Higher	Higher	Higher	Higher	Higher	Similar	Similar
In use Granules	Higher	Higher	Higher	Higher	Higher	Higher	Similar

* active in this phase

A similar approach can be applied to the impact of formulation on absorption from a spray solution. Absorption is dependant on the solubility of the active substance and the physical state of the active in the spray solution An example of the guidance is presented in Table 3.

Table 3: Dermal absorption of active substances from similar concentrations of Spray Solutions resulting from different formulation types.

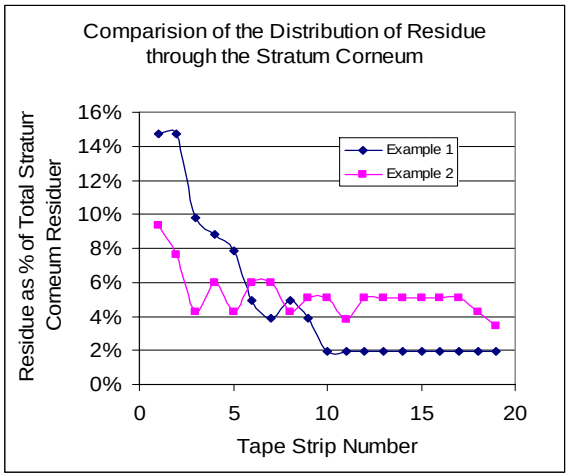
Registered Product: Formulation Type	New Formulation: EC	SL	Oil * /Water	Water*/Oil	SC/WP/WDG
Liquid	Similar	Similar	Similar	Similar	Similar
Solid	Similar	Similar	Similar	Similar	Similar
Conc. in spray solution, < than water solubility of active in water					
Solid	Higher	Higher	Higher	Higher	Similar
Conc. in spray dilution. > water solubility of active in water					

- active in this phase

No	Institution	Chapter	Stakeholder comment and PPR Panel response
			• An additional parameter that can impact on *read across* of dermal absorption values is the concentration, but this is also dependant on the physical state of the active, for example, solid or liquid, or the amount dissolved and/or suspended. Any significant differences in the physical state between differing concentrations can fundamental alter the availability of the active substance for absorption. Similarly a change in the vehicle (solvent) can have an impact on absorption. Therefore, the impact of differing concentration on *read across* has to be considered in terms of equivalent physical state of the active and the carrier vehicle.In an unpublished PSD review, the difference in dermal absorption for a range of actives ingredients from differing concentrations of spray dilutions indicated that for all actives, a 10-fold difference in concentration resulted in only a 2- to 3-fold increase in percentage absorption. Therefore it can be concluded that *read across* involving minor changes in formulation and spray dilution concentration would not require a pro-rated correction to be made to the experimentally derived values. *This issue is addressed in the new GD. See comment 78 (1)*
83	Dow Agrosciences, UK on behalf of the European Crop Protection Association (ECPA), Belgium	6. Comments for additional chapters	Stratum corneum- Tape stripping *in vivo* and *in vitro* studies OECD Guidance Document No. 28 describes how tapping stripping of the application site can be used to fractionated the amount of applied test substance residues in the different layers of the skin, specifically, the stratum corneum. This procedure is applicable to both *in vivo* and *in vitro* studies. The OECD guidance document mentions 15-20 tape strips for human skin. The number of tape strips taken determines the amount of detail provided on the distribution of the residue through the stratum corneum. Washing of the skin surface, both *in vivo* and *in vitro*, as required by the OECD test guidelines, leaves a proportion of the applied dose at the surface of the *stratum corneum*. This surface dose is not available for absorption and is readily removed by tape stripping. In effect, removal of this surface layer is equivalent to a washing procedure that mimics the efficiency of normal washing by an operator/worker. When 15 or more tape strips can be taken, at least the first two tape strips should be included with wash as non-absorbed dose. This has become an accepted procedure by many Member States. *In vivo* there are other options to determine if absorption from the application site (including the *stratum corneum*) has ceased (Section 3.2.1), but tape stripping can be useful to demonstrate potential movement of the residue within the *stratum corneum* over the duration of an *in vivo* study, which may provide additional information on the ultimate fate of the *stratum corneum* residue. *In vitro*, providing that the key aspects of OECD 428 have been followed, in particular, the ability of the test substance to partition freely into the receptor fluid, dermal absorption can be estimated from the receptor fluid alone. However, it is recognised that a proportion of the applied dose that remains within the skin sample following washing may have eventually diffused into the receptor fluid beyond the duration of the experiment. Although the normal process of desquamation removes chemicals lodged in the *stratum corneum in vivo* (Ramsey *et al*, 1992), in order to ensure the conservative nature of the *in vitro* approach, the proportion of the dose remaining in the skin preparation, in the absence of any additional information should be regarded as absorbed.

No	Institution	Chapter	Stakeholder comment and PPR Panel response

Data on the potential absorption from the *stratum corneum* can be obtained by examining the profile of the residue with in the *stratum corneum* by plotting the amount present against the tape strip number (Figure 1):



Example 1 demonstrates that movement through the stratum corneum has decreased to a minimum whilst Example 2 indicates that movement through the stratum corneum could be on going.

The *stratum corneum* data should also be considered in conjunction with the residue in the epidermis/dermis and the receptor fluid. The relationship between the stratum corneum residue and epidermis/dermis compared with the relationship between epidermis/dermis and receptor fluid can be used to determine if the *stratum corneum* will become available.

Guidance on such relationships and the outcome are given by the theoretical data in the following table, which contains three examples of receptor fluid values and three differing potential scenarios for *stratum corneum* and epidermis/dermis residues.

Example	SC	Epidermis/ dermis	Total skin	Receptor Fluid	Components in Absorbed Dose	Absorbed Dose
1a	>10	0.5	10.5	0.5	Epidermis/dermis plus receptor fluid	1
1b	5	5.5	10.5	0.5	Epidermis/dermis plus receptor fluid/part of the SC	6 +
1c	<2	8.5	10.5	0.5	Epidermis/dermis plus receptor fluid / SC excluding strips 1 and 2	9+
2a	>10	2.5	12.5	10	Epidermis/dermis	12.5

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					plus receptor fluid	
2b	5	5	10	10	Epidermis/dermis plus receptor fluid and part of the SC	15+
2c	<3	7	10	10	Epidermis/dermis plus receptor fluid / SC excluding strips 1 and 2	17+
3a	>10	2	12	25	Epidermis/dermis plus receptor fluid	27
3b	7	8	15	25	Epidermis/dermis plus receptor and part of the SC	33+
3c	<5	10	15	25	Epidermis/dermis plus receptor fluid / SC excluding strips 1 and 2	35+

SC Stratum corneum

Examples 1a, 2a and 3a clearly indicate that movement from the *stratum corneum* is negligible because levels in the epidermis are relatively low. Example 1c, 2c and 3c indicate a likelihood that the *stratum corneum* residue becomes systemically available with the exception of the surface layers. Guidance on the fate of the remaining *stratum corneum* can be obtained from examining the distribution of the residue through the *stratum corneum* (Figure 1). Examples 1b, 2b and 3b require expert judgment. A conservative approach that could be applied would be to exclude in all cases a minimum of the next 5 tape strips (i.e., 2 to7) as non-absorbed due to potential desquamation. In order to ensure the conservative nature of the *in vitro* approach, the proportion of the dose remaining in the skin preparation, in the absence of any additional information should be regarded as absorbed, unless there is clear evidence from the tape strip profile and/or the absorption profile or flux that absorption from the *stratum corneum* is negligible.

Trebilcock KL, Heylings JR and Wilks MF. *In vitro* tape stripping as a model for *in vivo* skin stripping. Toxicology *In vitro*, 8, 665-667, 1994.

Ramsey JD, Woollen BH, Auton TR, batten PL and Leeser JE. Pharmacokinetics of fluazifop butyl in human volunteers II: dermal dosing. Human and Experimental Toxicology, 11, 247-254, 1992.

Tape stripping and the relevance of the stratum corneum has been addressed extensively.

GLOSSARY

ADME study: Absorption, distribution, metabolism, excretion study.

Atopic dermatitis: A skin disease characterized by areas of severe itching, redness, scaling, and loss of the surface of the skin.

Area under the curve (AUC): Area under the plasma drug concentration versus time curve; a measure of drug exposure.

Dermal absorption: The movement of a chemical from the outer surface of the skin into the circulatory system leading to systemic exposure. Also called percutaneous absorption.

Dermal penetration: The movement of a chemical from the outer surface of the skin into the epidermis, but not necessarily into the circulatory system.

Dermis: The layer of cells below the epidermis. The dermis has a blood supply and sensory nerves and provides support for the epidermis.

Desquamation: The shedding of the superficial epithelium, as of skin, the mucous membranes, etc..

Epidermis: The outermost structure of the skin, consisting of several distinct cell layers.

Flux: The amount of material crossing a defined area in a set time. A chemical with a high dermal flux will be absorbed more readily than a chemical with a lower flux.

Full-thickness skin: Full-thickness skin preparations consist of a 500–1000 μm thick skin sample, incorporating the *stratum corneum*, viable epidermis, and dermis.

Kinetics: In the context of dermal absorption, how the absorption of a chemical changes over time.

Lag-phase: The time taken for the absorption of a chemical across the skin to reach a linear flux. Can be determined by extrapolating the line of linear flux back to the intercept at the X-axis of an absorption:time plot.

Log Pow: The logarithm of the relative maximum amount of a chemical that will dissolve in octanol and in water. A compound with a solubility of 100g/L in octanol and 1g/L in water would have a log Pow of 2.0.

Split-thickness skin: Split-thickness (dermatomed) skin consists of 200–400/500 μm thick sample, in which the lower dermis has been removed. A surgical instrument for cutting skin grafts, called dermatome, is used to obtain samples of uniform shape and thickness.

Stratum corneum: The outermost layer of the epidermis. Consists of several layers of non-viable cells (typically 15 – 20), the outermost cells are lost by sloughing off. Varies in thickness with anatomical site. Presents the major barrier to dermal absorption.

Tape stripping: A procedure performed at the end of a dermal absorption study that involves the application of adhesive tape to the area of skin that was exposed to a chemical. An even (often predetermined) pressure is applied to the tape before it is removed, taking a layer of *stratum corneum* cells with it. The tape strip is then analysed to determine the amount of chemical that was present in the removed *stratum corneum*. The process is repeated to remove sequentially lower layers of the *stratum corneum*.